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=> d his

     (FILE 'HOME' ENTERED AT 17:00:04 ON 03 DEC 1999)

     FILE 'MEDLINE, HCAPLUS, BIOSIS, USPATFULL' ENTERED AT 17:00:24 ON 03 DEC
     1999
L1           120 S SADELAIN M?/AU
L2           835 S CHEUNG N?/AU
L3           830 S KRAUSE A?/AU
L4          2685 S GUO H?/AU
L5             3 S L1 AND L2 AND L3 AND L4
L6          2453 S ANTI(W)GD2 OR GD2
L7         16286 S TRANSMEMBRANE(3A)(REGION OR DOMAIN)
L8         72510 S FUSION PROTEIN#
L9          7646 S CD-28 OR CD28 OR (CD 28)
L10           14 S L6 AND L9
L11            9 S L8 AND L10
L12            1 S L7 AND L11
L13            1 S L12 NOT L5
L14           13 S L10 NOT L13
L15           10 S L14 NOT L5
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appl search - 3 citations

1 citation

10 citations

=> d bib abs l13

L13 ANSWER 1 OF 1 USPATFULL
AN 1999:27746 USPATFULL
TI Tissue factor compositions and ligands for the specific coagulation of vasculature
IN Thorpe, Philip E., Dallas, TX, United States
Edgington, Thomas S., La Jolla, CA, United States
PA The Scripps Research Institute, La Jolla, CA, United States (U.S. corporation)
Board of Regents, The University of Texas System, Austin, TX, United States (U.S. corporation)
PI US 5877289 19990302
AI US 1995-479733 19950607 (8)
RLI Continuation-in-part of Ser. No. US 1994-273567, filed on 11 Jul 1994 which is a continuation-in-part of Ser. No. US 1994-205330, filed on 2 Mar 1994, now patented, Pat. No. US 5855866 which is a continuation-in-part of Ser. No. US 1992-846349, filed on 5 Mar 1992
DT Utility
EXNAM Primary Examiner: Feisee, Lila; Assistant Examiner: Bansal, Geetha P.
LREP Arnold White & Durkee L.L.P.
CLMN Number of Claims: 100
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 7148
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are various compositions and methods for use in achieving specific blood coagulation. This is exemplified by the specific in vivo coagulation of tumor vasculature, causing tumor regression, through the site-specific delivery of a coagulant using a bispecific antibody.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d kwic

L13 ANSWER 1 OF 1 USPATFULL
SUMM Alternatively, such bispecific coagulating agents may be **fusion proteins** prepared by molecular biological techniques, i.e., by joining a gene (or cDNA) encoding a binding ligand or region to a. . . segment encoding the coagulation factor and expressing the vector in a recombinant host cell so that it produces the encoded **fusion protein**.
SUMM . . . macrophages or mast cells; and those that bind to a tumor antigen and to the activation antigens CD2, CD3 or **CD28**, and preferably **CD28**, to stimulate IFN-.gamma. production by NK cells or preferably by T cells.
SUMM . . . cell surface activating antigen. Exemplary activating antigens include CD14 and CD16 (FcR for IgE) for monocytes/macrophages; and CD2, CD3 and **CD28** for T cells; with CD14 and **CD28**, respectively, being preferred for use in certain embodiments.
SUMM . . . method is to use a bispecific antibody that binds to both an effector cell activating antigen, such as CD14 or **CD28**, and to a disease or tumor cell antigen. These bispecific antibodies will localize to the disease or tumor site and. . .
DETD . . . Listing

renal cancer & glioblastomas	p155	6.1	Loop et al., 1981
bladder & laryngeal cancers	"Ca Antigen" 350-390 kD	CA1	Ashall et al., 1982
neuroblastoma	**GD2**	3F8	Cheung et al., 1986
Prostate	gp48 48 kD GP	4F.sub.7 /7A.sub.10	Bhattacharya et al., 1984

Prostate 60 kD GP 2C.sub.8 /2F.sub.7
. . .
DETD . . . bispecific antibodies such as these is predicated in part on the fact that cross-linking antibodies recognizing CD3, CD14, CD16 and **CD28** have previously been shown to elicit cytokine production selectively upon cross-linking with the second antigen (Qian et al., 1991). In. . .
DETD . . . macrophages
CD14
Molecule-1 Molecule-110
IL-1, TNF-
mast cells FcR for IgE
(INCAM-110)
.alpha.
(Immunoglobulin
TNF-.beta., IL-4
helper T cells
CD2, CD3, **CD28**
Family)
TNF NK cells FcR for IgG (CD16)
Intercellular
ICAM-1
-- IL-1, TNF.alpha.
monocytes CD14
Adhesion (Immunoglobulin
(Bacterial
macrophages
CD15
Molecule-1 Family) Endotoxin)
mast cells FcR for IgE
TNF-.beta.,
T helper cells
CD2, CD3, **CD28**
IFN.gamma.
NK cells FcR for IgG (CD16)
The Agent for
LAM-1 MEL-14 Agent
Il-1, TNF.alpha.
monocytes CD14
Leukocyte Agent (Mouse) (Bacterial
macrophages
CD14
Adhesion Endotoxin)
mast cells FcR for IgE
Molecule-1
Major MHC HLA-DR
Human IFN-.gamma.
helper T cells
CD2, CD3, **CD28**
Histocompatabi
Class HLA-DP
lity Complex
II HLA-DQ
Class II
Antigen I-A
Mouse NK cells
FcR for IgG (CD16)
I-E

DETD . . . receptor) and FcR for IgE, which will activate the release of IL-1 and TNF.alpha.; and CD16, CD2 or CD3 or **CD28**, which will activate the release of IFN.gamma. and TNF.beta., respectively.
DETD . . . for IgE, found on Mast cells; FcR for IgG (CD16), found on NK cells; as well as CD2, CD3 or **CD28**, found on the surfaces of T cells. Of these, CD14 targeting is generally preferred due to the relative prevalence of. . .
DETD . . . the other cytokines. Thus, for the practice of this aspect of the invention, one will desire to select CD2, CD3, **CD28**, or most preferably **CD28**, as the cytokine activating antigen for targeting by the antigen-inducing bispecific antibody.

DETD . . . use of a bispecific (Fab'--Fab') antibody having one arm directed against a tumor antigen and the other arm directed against **CD28** is currently preferred. This antibody will crosslink **CD28** antigens on T cells in the tumor which, when combined with a second signal (provided, for example, by IL-1 which. . .

DETD . . . various cytokine activating molecules is also well known in the art. For example, the preparation and use of anti-CD14 and anti-**CD28** monoclonal antibodies having the ability to induce cytokine production by leukocytes has now been described by several laboratories (reviewed in. . .

DETD . . . more suitable for the MHC Class II approach involving, e.g., the cross-linking of T cells in the tumor through an anti-**CD28** /anti-tumor bispecific antibody, because these tumors are more likely to be infiltrated by T cells, a prerequisite for this method to. . .

DETD 2. Recombinant **Fusion Proteins**

DETD The bispecific targeted coagulants of the invention may also be **fusion proteins** prepared by molecular biological techniques. The use of recombinant DNA techniques to achieve such ends is now standard practice to. . .

DETD When produced via recombinant DNA techniques, the targeting agent/coagulating agent compounds of the invention are referred to as "**fusion proteins**". It is to be understood that such **fusion proteins** contain, at least, a targeting agent and a coagulating agent as defined in this invention, and that the agents are operatively attached. The **fusion proteins** may also include additional peptide sequences, such as peptide spacers which operatively attach the targeting agent and coagulating agent compounds, as long as such additional sequences do not appreciably affect the targeting or coagulating activities of the resultant **fusion protein**.

DETD . . . contemplated to be a significant problem, however, those of skill in the art will know to confirm that a recombinant **fusion protein** functions as intended, and expected from other data, before use in a clinical setting.

DETD . . . example, when large quantities of bispecific agent are to be produced, vectors that direct the expression of high levels of **fusion protein** products that are readily purified may be desirable. Such vectors include, but are not limited to, the E. Coli expression. . . coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a **fusion protein** additionally containing a portion of the lac Z product is provided; pIN vectors (Inouye et al., 1985; Van Heeke et. . . the like. pGEX vectors may also be used to express foreign polypeptides, such as the targeting agent/coagulating agent combinations as **fusion proteins** additionally containing glutathione S-transferase (GST). In general, such **fusion proteins** are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the. . . are designed to include thrombin or factor Xa protease cleavage sites so that the binding agent/coagulant protein of the overall **fusion protein** can be released from the GST moiety.

DETD . . . length human TF, the inventors used a truncated form (tTF), which is devoid of the cytoplasmic as well as the **transmembrane domain**. Truncated TF lacks coagulation inducing activity, while still being able to complex factor VIIa, probably because it is not able. . .

DETD . . . pharmaceutical composition that comprises a bispecific antibody that binds to the activating antigen CD14, CD16 (FcR for IgE), CD2, CD3, **CD28** or the T-cell receptor antigen are preferred, with CD14 or **CD28** binding bispecific antibodies being more preferred. Activation of monocyte/macrophages or mast cells via CD14 or CD16 binding results in IL-1 production that induces E-selectin; whereas activation of T cells via CD2, CD3 or **CD28** binding results in IFN-.gamma. production that induces MHC class II. Kits that include a second pharmaceutical composition that comprises a. . .

DETD Method I: Expression, Refolding and Purification of tTF from E. coli. The poly(his)-tTF **fusion protein** was expressed using BL21 cells transformed with pTrc-HisC-tTF. Inoculant cultures (10 ml in LB medium) were grown overnight shaking at. . .

DETD Ni--NTA column fractions containing the **fusion protein**

were combined and dithiothreitol was added to 50 mM. The solution was held at room temperature overnight then diluted to. . .

DETD . . . structures: tTF.sub.1-219 (X).sub.n2 (Y).sub.n2 Z Ligand, where tTF.sub.1-219 represents TF minus the cytosolic and transmembrane domains; X represents a hydrophobic **transmembrane domain** n1 amino acids (AA) in length (1-20 AA); Y represents a hydrophilic protease recognition sequence of n2 AA in length. . .

CLM What is claimed is:

65. The binding ligand of claim 10, wherein said binding ligand is a **fusion protein** prepared by expressing a recombinant vector in a host cell, wherein the vector comprises, in the same reading frame, a. . .

=> d bib abs 15

L5 ANSWER 1 OF 3 MEDLINE
AN 1998372753 MEDLINE
DN 98372753
TI Antigen-dependent CD28 signaling selectively enhances survival and proliferation in genetically modified activated human primary T lymphocytes.
AU **Krause A; Guo H F;** Latouche J B; Tan C; **Cheung N K; Sadelain M**
CS Department of Human Genetics, Memorial Sloan-Kettering Cancer Center, New York 10021, USA.
NC CA-08748 (NCI)
SO JOURNAL OF EXPERIMENTAL MEDICINE, (1998 Aug 17) 188 (4) 619-26.
Journal code: I2V. ISSN: 0022-1007.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 19981?
EW 19981202
AB Most tumor cells function poorly as antigen-presenting cells in part because they do not express costimulatory molecules. To provide costimulation to T lymphocytes that recognize tumor cells, we constructed a CD28-like receptor specific for GD2, a ganglioside overexpressed on the surface of neuroblastoma, small-cell lung carcinoma, melanoma, and other human tumors. Recognition of GD2 was provided by a single-chain antibody derived from the GD2-specific monoclonal antibody 3G6. We demonstrate that the chimeric receptor 3G6-CD28 provides CD28 signaling upon specific recognition of the GD2 antigen on tumor cells. Human primary T lymphocytes retrovirally transduced with 3G6-CD28 secrete interleukin 2, survive proapoptotic culture conditions, and selectively undergo clonal expansion in the presence of an antiidiotypic antibody specific for 3G6-CD28. Polyclonal CD8(+) lymphocytes expressing 3G6-CD28 are selectively expanded when cultured with cells expressing allogeneic major histocompatibility complex class I together with GD2. Primary T cells given such an antigen-dependent survival advantage should be very useful to augment immune responses against tumor cells.

=> d bib abs l5 2

L5 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 1999 ACS
AN 1998:554622 HCAPLUS
DN 129:259130
TI Antigen-dependent CD28 signaling selectively enhances survival and proliferation in genetically modified activated human primary T lymphocytes
AU **Krause, Anja; Guo, Hong-Fen;** Latouche, Jean-Baptiste; Tan, Cuiwen; **Cheung, Nai-Kong V.; Sadelain, Michel**
CS Department of Human Genetics, Memorial Sloan-Kettering Cancer Center, NY, 10021, USA
SO J. Exp. Med. (1998), 188(4), 619-626
CODEN: JEMEAV; ISSN: 0022-1007
PB Rockefeller University Press
DT Journal
LA English
AB Most tumor cells function poorly as antigen-presenting cells in part because they do not express costimulatory mols. To provide costimulation to T lymphocytes that recognize tumor cells, we constructed a CD28-like receptor specific for GD2, a ganglioside overexpressed on the surface of neuroblastoma, small-cell lung carcinoma, melanoma, and other human tumors. Recognition of GD2 was provided by a single-chain antibody derived from the GD2-specific monoclonal antibody 3G6. We demonstrate that the chimeric receptor 3G6-CD28 provides CD28 signaling upon specific recognition of the GD2 antigen on tumor cells. Human primary T lymphocytes retrovirally transduced with 3G6-CD28 secrete interleukin 2, survive proapoptotic culture conditions, and selectively undergo clonal expansion in the presence of an antiidiotypic antibody specific for 3G6-CD28. Polyclonal CD8+ lymphocytes expressing 3G6-CD28 are selectively expanded when cultured with cells expressing allogeneic major histocompatibility complex class I together with GD2. Primary T cells given such an antigen-dependent survival advantage should be very useful to augment immune responses against tumor cells.

=> d bib abs l5 3

L5 ANSWER 3 OF 3 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1998:448897 BIOSIS
DN PREV199800448897
TI Antigen-dependent CD28 signaling selectively enhances survival and proliferation in genetically modified activated human primary T lymphocytes.
AU **Krause, Anja; Guo, Hong-Fen;** Latouche, Jean-Baptiste; Tan, Cuiwen; **Cheung, Nai-Kong V.; Sadelain, Michel (1)**
CS (1) Box 182, Memorial Sloan-Kettering Cancer Cent., 1275 York Ave., New York, NY 10021 USA
SO Journal of Experimental Medicine, (Aug. 17, 1998) Vol. 188, No. 4, pp. 619-626.
ISSN: 0022-1007.
DT Article
LA English
AB Most tumor cells function poorly as antigen-presenting cells in part because they do not express costimulatory molecules. To provide costimulation to T lymphocytes that recognize tumor cells, we constructed a CD28-like receptor specific for GD2, a ganglioside overexpressed on the surface of neuroblastoma, small-cell lung carcinoma, melanoma, and other human tumors. Recognition of GD2 was provided by a single-chain antibody derived from the GD2-specific monoclonal antibody 3G6. We demonstrate that the chimeric receptor 3G6-CD28 provides CD28 signaling upon specific recognition of the GD2 antigen on tumor cells. Human primary T lymphocytes retrovirally transduced with 3G6-CD28 secrete interleukin 2, survive proapoptotic culture conditions, and selectively undergo clonal expansion in the presence of an antiidiotypic antibody specific for 3G6-CD28. Polyclonal CD8+ lymphocytes expressing 3G6-CD28 are selectively expanded when cultured with cells expressing allogeneic major histocompatibility complex class I together with GD2. Primary T cells given such an antigen-dependent survival advantage should be very useful to augment immune responses against tumor cells.

=> d bib abs kwic l15

L15 ANSWER 1 OF 10 HCAPLUS COPYRIGHT 1999 ACS
AN 1997:267109 HCAPLUS
DN 126:250223
TI Antibodies with two or more specificities for the selective elimination of tumor cells in vivo
IN Lindhofer, Horst; Thierfelder, Stefan
PA GSF - Forschungszentrum fuer Umwelt und Gesundheit Gmbh, Germany
SO Ger. Offen., 17 pp.
CODEN: GWXXBX
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 19531348	A1	19970227	DE 1995-19531348	19950825
	WO 9708205	A1	19970306	WO 1996-EP3734	19960823

W: US
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

PRAI DE 1995-19531348 19950825
AB Antibodies are disclosed which have two or more specificities, one a tumor-assocd. antigen, and the other a non-tumor-sp. surface antigen, such as CD antigens. The antibodies can be used for the immunotherapy of tumors such as B-cell lymphoma, colorectal carcinoma, melanoma, ovarian carcinoma, glioblastoma, or mammary carcinoma. Thus, multi-specific antibodies were prepd. and used to selectively deplete tumor cells in vivo.
IT 17-1A antigen
Antigens
CA 125 (carbohydrate antigen)
CD1 (antigen)
CD14 (antigen)
CD19 (antigen)
CD2 (antigen)
CD20 (antigen)
CD22 (antigen)
CD28 (antigen)
CD3 (antigen)
CD4 (antigen)
CD40 (antigen)
CD45 (antigen)
CD5 (antigen)
CD7 (antigen)
CD8 (antigen)
Class II MHC antigens
Complement receptor type 2
Fc.gamma.RI receptors
Fc.gamma.RII receptors
Fc.gamma.RIII receptors
Fc.epsilon.RII receptors
Idiotypes (immunoglobulin/TCR)
Tumor-associated antigen
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(multispecific antibodies to tumor-assocd. antigens and cell surface antigens for selective elimination of tumor cells in vivo)
IT 9054-63-1, Antigens, CD13 62010-37-1, Ganglioside GD3 65988-71-8, Ganglioside **GD2** 82707-54-8, Neprilysin
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(multispecific antibodies to tumor-assocd. antigens and cell surface antigens for selective elimination of tumor cells in vivo)

=> d bib abs kwic l15 2

L15 ANSWER 2 OF 10 USPATFULL
AN 1999:137464 USPATFULL
TI Monoclonal antibody 1A7 and related polypeptides
IN Chatterjee, Malaya, Lexington, KY, United States
Foon, Kenneth A., Lexington, KY, United States
Chatterjee, Sunil K., Lexington, KY, United States
PA The Board of Trustees of the University of Kentucky, Lexington, KY, United States (U.S. corporation)
PI US 5977316 19991102
AI US 1996-591196 19960116 (8)
RLI Continuation-in-part of Ser. No. US 1995-372676, filed on 17 Jan 1995, now patented, Pat. No. US 5612030
DT Utility
EXNAM Primary Examiner: Huff, Sheela; Assistant Examiner: Reeves, Julie E.
LREP Morrison & Foerster LLP
CLMN Number of Claims: 32
ECL Exemplary Claim: 1
DRWN 22 Drawing Figure(s); 22 Drawing Page(s)
LN.CNT 4578
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to monoclonal antibody 1A7. This is an anti-idiotype produced by immunizing with an antibody specific for ganglioside **GD2**, and identifying a hybridoma secreting antibody with immunogenic potential in a multi-step screening process. Also disclosed are polynucleotide and polypeptide derivatives based on 1A7, including single chain variable region molecules and fusion proteins, and various pharmaceutical compositions. When administered to an individual, the 1A7 antibody overcomes immune tolerance and induces an immune response against **GD2**, which comprises a combination of **anti-GD2** antibody and **GD2**-specific T cells. The invention further provides methods for treating a disease associated with altered **GD2** expression, particularly melanoma, neuroblastoma, glioma, soft tissue sarcoma, and small cell carcinoma. Patients who are in remission as a result of traditional modes of cancer therapy may be treated with a composition of this invention in hopes of reducing the risk of recurrence.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB . . . present invention relates to monoclonal antibody 1A7. This is an anti-idiotype produced by immunizing with an antibody specific for ganglioside **GD2**, and identifying a hybridoma secreting antibody with immunogenic potential in a multi-step screening process. Also disclosed are polynucleotide and polypeptide. . . various pharmaceutical compositions. When administered to an individual, the 1A7 antibody overcomes immune tolerance and induces an immune response against **GD2**, which comprises a combination of **anti-GD2** antibody and **GD2**-specific T cells. The invention further provides methods for treating a disease associated with altered **GD2** expression, particularly melanoma, neuroblastoma, glioma, soft tissue sarcoma, and small cell carcinoma. Patients who are in remission as a result. . .
SUMM . . . that gangliosides may be preferable to other types of target antigens for antibody-mediated killing of certain tumor types. Gangliosides like **GD2** have simple, well-defined structures, and the level of expression is not affected by antibody binding. In vitro studies have shown that monoclonal antibodies against gangliosides like **GD2** and GD3 potentiate lymphocyte response which could potentially be directed towards tumor cells. In addition, certain gangliosides have been implicated. . .
SUMM In particular, glycosphingolipid **GD2** is expressed at high density by tumors of human neuroectodermal origin; including malignant melanoma, neuroblastoma, glioma, soft tissue sarcoma and small cell carcinoma of the lung. The **GD2** antigen is absent in most normal tissues, except for low levels in brain and peripheral nerve.
SUMM . . . the cancers for which gangliosides hold significant promise as a target antigen (Livingston (1995) Immunol. Rev. 145:147-166).

Increased expression of **GD2** has been observed in a majority of malignant melanoma cells. Several murine monoclonal **anti-GD2** antibodies were reported to suppress the growth of tumors of neuroectodermal origin in athymic (nu/nu) mice or cause remission in patients with metastic melanoma. A human-mouse chimeric **anti-GD2** antibody remissions in patients with metastic neuroblastoma. The mechanism is thought to involve antibody dependent cellular cytotoxicity (ADCC) or complement-mediated cytotoxicity (CMC). Clinical responses have been obtained by treating with monoclonal antibodies against GM2, **GD2** and GD3. Active immunization with a ganglioside vaccine comprising GM2 produced anti-GM2 antibodies in 50/58 patients, who survived longer on. . .

SUMM If there was a simple and reliable therapeutic strategy for providing immune reactivity against **GD2**, then the clinical prospects for these types of cancers might improve.

SUMM Unfortunately, there are several reasons why **GD2** is less than ideal as a component of an active vaccine. For one thing, **GD2** is of limited supply, and is difficult to purify. Of course, because **GD2** is a ganglioside, it cannot be generated by simple recombinant techniques. Secondly, gangliosides in general, and **GD2** in particular, are poorly immunogenic. In order to render them more immunogenic in humans, it has been necessary to conjugate. . .

SUMM Similarly, the passive administration of **anti-GD2** antibodies is less than ideal as an approach to long-term care. The amount of antibody that must be provided passively. . .

SUMM How else, then, could an active immune response against **GD2** be obtained? The network hypothesis of Lindemann and Jerne suggests a way of overcoming both the natural immune tolerance against **GD2**, and the shortage of supply of **GD2**. It relies on the fact that antibodies comprise variable region epitopes that themselves may be immunogenic, leading to the generation. . .

SUMM . . . (Kanda et al., Yamamoto et al., Hastings et al.). Saleh et al. and Cheung et al. have raised anti-idiotypes against **GD2**. Other anti-idiotypes have entered early clinical tials: for example, Mittelman et al. are using an anti-idiotype related to a high. . .

SUMM . . . disclosure outlines a particular monoclonal anti-idiotype antibody, designated 1A7. This antibody has been established as being capable of eliciting an **anti-GD2** response. It has all the desirable properties that provide for escaping immune tolerance to **GD2**, and is appropriate for treating **GD2** -associated disease.

SUMM Yet another embodiment is a method of treating a **GD2** -associated disease in an individual, comprising administering monoclonal antibody 1A7, or a polynucleotide or polypeptide of this invention. The disease may. . .

SUMM A further embodiment of this invention is a kit for detection or quantitation of an **anti-GD2** antibody or a 1A7 polynucleotide in a sample, comprising monoclonal antibody 1A7 or a polynucleotide or polypeptide of this invention. . .

DRWD FIG. 6 is a bar graph depicting inhibition of binding of .sup.125 I labeled 14G2a antibody to **GD2** positive melanoma cell line M21/P6 in the presence of different concentrations of Ab1 and monkey Ab3. Parallel inhibition curves were. . .

DRWD . . . from a FACS analysis of the binding of monkey Ab3 to tumor cells. Panel A shows the staining observed of **GD2**-expressing M21/P6 cells labeled with preimmune and immune Ab3. Panel B shows the staining observed on another cell line not expressing **Gd2**. Panel C shows control staining of M21/P6 cells using the **GD2** -specific antibody 14G2a, or no antibody.

DRWD . . . hand (solid) bar denotes the binding of Ab3 from monkey PRO#685; the right hand (hatched) bar denotes control binding by **anti-GD2** 14G2a. This experiment shows the antibody induced upon immunization with the anti-idiotype 1A7 is antigen specific.

DRWD FIG. 9 is a bar graph depicting inhibition of binding of .sup.125 I-labeled 14G2a antibody to purified **GD2** by 14G2a and monkey Ab3. For each triad of bars, the left hand (solid) bar denotes monkey PRO#778; the middle. . .

DETD We have discovered an anti-idiotype antibody that is capable of

recruiting a tumor-specific response against **GD2**. The antibody is designated 1A7. The immune response elicited by 1A7 typically comprises both humoral and cellular components, and is therefore expected to be useful in palliating the clinical conditions related to **GD2**-associated tumors. The invention comprises the 1A7 antibody molecule, along with polynucleotide and polypeptide derivatives thereof, and methods for using these. . .

DETD Cancer patients are typically tolerized to various tumor associated antigens (TAA), including **GD2**. 1A7 successfully circumvents immune tolerance, and elicits an immune response against **GD2**. According to the network theory, Ab1 represents anti-tumor monoclonal antibody; Ab2 represents anti-idiotypic monoclonal antibody; and Ab3 represents anti-anti-idiotypic monoclonal. . .

DETD . . . one explanation is that the 1A7 combining site may present a region that at least partly resembles an epitope in **GD2** in the context of one or more other epitopes which render it more immunogenic. The epitope of **GD2** which may resemble that of 1A7 is identified by the Ab1 (14G2a) used to generate 1A7. As a result, 1A7 escapes the normal immune tolerance against **GD2**, and is able to elicit an **anti-GD2** response.

DETD The 1A7 antibody and derivatives thereof are useful, for example, for eliciting an **anti-GD2** immune response, for treating a **GD2**-associated disease, and as reagents for detecting the presence of **anti GD2**.

DETD . . . and treatment modalities of this invention may be brought to bear whenever it is desirable to elicit a response against **GD2** , especially in humans. Human patients with **GD2**-associated tumors, including melanoma, neuroblastoma, glioma, soft tissue sarcoma, and small cell carcinoma (including small cell lung cancer) are especially appropriate. . .

DETD "1A7" is a particular anti-idiotype antibody raised against the **anti-GD2** monoclonal antibody with the designation 14G2a. The generation and characterization of 1A7 is described in Example 1.

DETD . . . activities: ability to specifically bind monoclonal antibody 14G2a; ability to inhibit the binding of 1A7 to 14G2a or 14G2a to **GD2** in a specific manner; and an ability to elicit an immune response against **GD2**. A specific immune response may comprise antibody, B cells, T cells, and any combination thereof, and effector functions resulting therefrom.. . . other biological activity ascribed to 1A7 in this disclosure, including the role of 1A7 in the amelioration or palliation of **GD2**-associated disease.

DETD . . . following properties: ability to bind monoclonal antibody 14G2a; ability to inhibit the binding of 1A7 to 14G2a or 14G2a to **GD2** in a specific manner; and an ability to elicit an immune response with a similar antigen specificity as that elicited. . .

DETD . . . reactive against the target, or any combination thereof. For purposes of this invention, the target is primarily tumor associated antigen **GD2**, but also includes any tumor associated antigen bound by 14G2a. The immunological reactivity may be desired for experimental purposes, for. . .

DETD . . . invention, an effective amount of a 1A7 polynucleotide or polypeptide is an amount that induces an immune response, particularly an **anti-GD2** response. In terms of treatment, an effective amount is amount that is sufficient to palliate, ameliorate, stabilize, reverse or slow the progression of the **GD2** -associated disease, or otherwise reduce the pathological consequences of the disease.

DETD 1A7 was obtained by immunizing naive mice with 14G2a **anti-GD2** antibody to obtain an anti-idiotype response. 14G2a binds to a unique epitope of **GD2**. Syngeneic BALB/c mice were immunized four times with 14G2a (Ab1) and their spleen cells were fused with the non-secretory mouse. . .

DETD . . . against antibody recognizing isotypic or allotypic determinants; (3) Positive selection for an ability to inhibit the binding of 14G2a to **GD2**; and (4) Positive selection for an ability to induce a humoral immune response against the original tumor-associated antigen (**GD2**) in both mice and rabbits.

DETD . . . To determine whether the anti-1 4G2a were directed against the paratope of 14G2a, the binding of radiolabeled 14G2a to the **GD2**

-positive cell line M21/P6 was studied in the presence of varying amounts of Ab2 hybridoma culture supernatants. With as little as. . .

DETD . . . serum samples were titered for the presence of Ab3 that bound not only to the immunizing Ab2, but also to **GD2**. The Ab2 passing all of these screening stages was designated 1A7. Further details of the method used to obtain 1A7. . .

DETD . . . 1A7 has been further characterized. The immune sera from both mice and rabbits competed with 14G2a for binding to the **GD2** -associated cell line M21/P6 and inhibited the binding of radioiodinated 14G2a to 1A7. This indicated that anti-anti-Id (Ab3) in mice and. . . epitope as Ab1. Administration of 1A7 to non-human primates (cynomolgus monkeys) also generated a specific immune response, comprising activity against **GD2** (Example 3).

DETD . . . capable of producing a detectable signal. These conjugated antibodies are useful, for example, in detection systems such as quantitation of **anti-GD2** or tumor imaging. Such labels are known in the art and include, but are not limited to, radioisotopes, enzymes, fluorescent. . .

DETD The 1A7 antibody may be used for a number of purposes. These include eliciting an antibody response to 1A7 or **GD2**, eliciting a T cell response to 1A7 or **GD2**, and treating various types of cancer. These uses are elaborated more fully in a later section.

DETD 1A7 may also be used to purify anti-1A7 (Ab3), **anti-GD2** (Ab1'), or 14G2a (Ab1). The method comprises contacting a biological sample containing the antibody with a 1A7 polypeptide, producing a. . .

DETD The invention also encompasses methods of detecting anti-1A7 or **anti-GD2** in a biological sample. **Anti-GD2** is detectable whenever (like 14G2a) it cross-reacts with 1A7. **Anti-GD2** with this activity may spontaneously arise during the course of a **GD2**-associated disease. **Anti-GD2** with this activity is especially likely in individuals who have received a course of therapy with 1A7, or a derivative. . . for monitoring antibody levels in an individual, as well as an industrial setting, in which commercial production of anti-1A7 or **anti-GD2** is desired.

DETD The assay methods entail contacting any anti-1A7 or **anti-GD2** target antibody in the sample with a 1A7 antibody or polypeptide under conditions suitable to allow the formation of a. . .

DETD Antibody assays may be conducted entirely in fluid phase. For example, **anti-GD2** may be mixed with labeled 1A7. Alternatively, the **anti-GD2** in the sample may be used to compete with a labeled **anti-GD2** for binding sites on 1A7. Generally, bound and unbound label is separated to quantitate the percent bound. Suitable separation methods. . .

DETD . . . anti-immunoglobulin. In this assay, the amount of label associated with the solid phase is inversely related to the amount of **anti-GD2** in the sample.

DETD . . . target antibody is captured by 1A7 attached directly or through a secondary reagent to a solid phase. After washing, the **anti-GD2** is detected using anti-immunoglobulin of the appropriate species, or a second 1A7 antibody, to which a label is directly or. . . In this type of assay, the amount of label associated with the solid phase correlates positively with the amount of **anti-GD2** in the sample. Other methods of measuring specific antibody are known in the art, and may be adapted to measure anti-1A7 or **anti-GD2** by using 1A7 as the specific reagent.

DETD 1A7 may also be used to measure the level of cellular anti-1A7 or **anti-GD2** activity. In one example, 1A7 is used to identify **anti-GD2** expressing cells in a cell suspension, perhaps B or T lymphocytes expressing a receptor that binds 1A7. 1A7 may be. . . or protein A. Suitable labels for this purpose include radiolabels and fluorescent labels. The use of fluorescent labels also allows **anti-GD2** cells to be separated from non-specific cells in a fluorescence-activated cell sorter. In a second example, **anti-GD2** expressing cells are detected in a tissue section. Typically, the tissue is fixed and embedded in a suitable medium, overlaid. . .

DETD . . . polypeptides encoded thereby. These functionally equivalent variants, derivatives, and fragments display the ability to induce an

immune response, preferably an **anti-GD2** immune response. For instance, changes in a DNA sequence that do not change the encoded amino acid sequence, as well. . .

DETD . . . altering cells in vivo. The purpose may include (but is not limited to) eliciting an antibody response to 1A7 or **GD2**, eliciting a T cell response to 1A7 or **GD2**, and treating various types of cancer. These uses are elaborated more fully in a later section.

DETD . . . pair of light and heavy chains is from 1A7. In one example, each light-heavy chain pair binds different epitopes of **GD2**. Such hybrids may also be formed using chimeric heavy or light chains.

DETD . . . Constructs wherein the 1A7 polypeptide is linked directly to particle-forming protein coding sequences produce hybrids which are immunogenic for an **anti-GD2** response. The vectors also comprise immunogenic HBV epitopes; for example, the pre-S peptide and stimulate a response against HBV. Such. . .

DETD . . . are conjugated to a carrier molecule. This is desirable for a 1A7 peptide that comprises a suitable epitope for eliciting **anti-GD2**, but is too small to be immunogenic. Any conjugation method known in the art may be used. Any carrier can. . .

DETD . . . inhibit the binding between 14G2a and intact 1A7, or for its ability to specifically inhibit the binding between 14G2a and **GD2**. Alternatively, a 1A7 polypeptide can be tested for its ability to elicit an immune response, preferably an **anti-GD2** response. 1A7 polypeptides can also be tested for their ability to palliate or ameliorate **GD2**-associated disease, such as **GD2**-associated tumors. It is understood that only one of these properties need be present in order for a polypeptide to come. . .

DETD . . . the putative 1A7 polypeptide is titered for its ability to decrease the binding of 1A7 to 14G2a, or 14G2a to **GD2**. Either of the binding pairs in the reaction to be inhibited is labeled, while the other is typically insolubilized in order to facilitate washing. **GD2**, if it is used, may be provided as the purified ganglioside, or as a **GD2**-expressing cell line, like M21/P6. The 1A7 polypeptide is typically mixed with the labeled component, and then the mixture is combined. . .

DETD Preferred uses of these compounds include eliciting an antibody response to 1A7 or more preferably **GD2**, eliciting a T cell response to 1A7 or more preferably **GD2**, and treating various types of **GD2**-associated cancer. These uses are elaborated more fully in a later section.

DETD . . . 1A7 either alone or in combination. Such pharmaceutical compositions and vaccines are useful for eliciting an immune response and treating **GD2**-associated diseases, either alone or in conjunction with other forms of therapy, such as chemotherapy or radiotherapy.

DETD . . . response. Since the objective is typically to identify compositions useful in cancer therapy, the samples are preferably measured for an **anti-GD2** response, as manifest in direct or inhibition type experiments.

DETD Presence of anti-1A7 (Ab3) and **anti-GD2** (Ab1') activity in a humoral response is preferably determined after first pre-incubating sera with autologous immunoglobulin or adsorbing on a. . . Results from this assay are compared to those obtained before administration of the 1A7 polypeptide (Example 1). Alternatively, binding to **GD2** positive cells, such as. M21/P6 cells, can be tested using immune flow cytometry. In a third example, the specificity of Ab3 is determined by Western blot. **GD2** is separated by SDS-PAGE and blotted to a nitrocellulose filter. The filter is then incubated with sera containing Ab3, and the reaction developed by a suitably labeled anti-immunoglobulin. If the Ab3 binds to **GD2**, a band at the appropriate molecular weight should appear.

DETD . . . that Ab3 and 14G2a contain at least some similar binding determinants. Competition of Ab3 with the binding of 14G2a to **GD2** may also be measured.

DETD . . . its ability to elicit an antibody that is cytotoxic. For determination of complement mediated cytotoxicity (CMC), M21/P6 target cells expressing **GD2** are labeled with .sup.51 Cr. Labeling may be accomplished by incubating about 10.sup.6 cells with approximately

200 .mu.Ci Na.sub.2 SO.sub.4. . .

DETD Another way of characterizing a composition of this invention is by testing its ability to elicit an **anti-GD2** antibody that participates in an ADCC response (Cheresh et al. (1986) Cancer Research 46:5112-5118). In this assay, cultured human M21/P6 cells (which express **GD2** in their surface) are labeled with .sup.51 Cr and are used as target cells. Normal human peripheral blood mononuclear cells. . .

DETD . . . assay (Kantor et al. (1992) J. Natl. Cancer Inst. 84:1084-1091). An example of a 51Cr release assay is the following. **GD2**-positive tumor cells (typically 1-2.times.10.sup.6 cells) are radiolabeled as target cells with about 200 .mu.Ci of Na.sub.2 .sup.51 CrO.sub.4 (Amersham Corp.,. . .

DETD . . . way of characterizing a 1A7 polypeptide is testing its ability to ameliorate, delay the progression or reduce the extent of **GD2** -associated disease, as outlined in the following section.

DETD . . . may be used for administration to individuals. They may be administered for experimental purposes, or to obtain a source of **anti-GD2**.

DETD Compositions of this invention are particularly suitable for administration to human individuals with a **GD2**-associated disease. A **GD2** associated disease is one in which expression of the **GD2** ganglioside is altered at the affected tissue site, usually an elevation in cell-surface expression. Relevant diseases are those in which an active immune response against **GD2** would confer a clinical benefit. Especially relevant are **GD2** -associated cancers; particularly melanoma, neuroblastoma, glioma, sarcoma, and small cell lung cancer.

DETD . . . this invention may be used to elicit an immune response. This includes an anti-1A7 specific response, and more preferably an **anti-GD2** response. The desired response may be a specific antibody response; a specific T helper-inducer repines, or a specific cytotoxic T. . .

DETD Also included in this invention are methods for treating **GD2** -associated disease, such as a tumor expressing **GD2**. The method comprises administering an amount of a pharmaceutical composition effective to achieve the desired effect, be it palliation of. . .

DETD For treatment of a **GD2**-associated disease in vivo, the amount of a pharmaceutical composition administered is an amount effective in producing the desired effect. An. . .

DETD Suitable subjects include those who are suspected of being at risk of a pathological effect of any **GD2**-associated condition are suitable for treatment with the pharmaceutical compositions of this invention. Those with a history of a **GD2**-associated cancer are especially suitable.

DETD . . . insufficient to identify this population). A pharmaceutical composition embodied in this invention is administered to these patients to elicit an **anti-GD2** response, with the objective of palliating their condition. Ideally, reduction in tumor mass occurs as a result, but any clinical. . .

DETD . . . subjects is known in the art as the "adjuvant group". These are individuals who have had a history of a **GD2**-associated cancer, but have been responsive to another mode of therapy. The prior therapy may have included (but is not restricted. . .

DETD . . . or after the initial treatment. These features are known in the clinical arts, and are suitably defined for each different **GD2** -associated cancer. Features typical of high risk subgroups are those in which the tumor has invaded neighboring tissues, or who show. . .

DETD . . . invention is administered to patients in the adjuvant group, or in either of these subgroups, in order to elicit an **anti-GD2** response. Ideally, the composition delays recurrence of the cancer, or even better, reduces the risk of recurrence (i.e., improves the. . .

DETD . . . treatment of cells ex vivo. This may be desirable for experimental purposes, or for treatment of an individual with a **GD2**-associated disease. In one example, the 1A7 antibody, or a polynucleotide or polypeptide derivative are administered to a culture of cells,. . .

DETD . . . 1A7 antibodies and polypeptide derivatives to remove a label (particularly a radiolabel) from an individual who has received a

labeled **anti-GD2** antibody (such as 14G2a) in the course of radioscintigraphy or radiotherapy. Effective imaging using radiolabeled antibodies is hampered due to. . . 1A7 antibody or a polypeptide derivative is administered to the individual at a specified time after administration of the labeled **anti-GD2**.

The intention is for the 1A7 polypeptide to complex with **anti-GD2** at sites other than the tumor, such as in the circulation and interstitial spaces, and thereby promote its clearance. As. . . is desirable to reduce collateral exposure of unaffected tissue. This invention thus includes methods of treatment in which a radiolabeled **anti-GD2** antibody is administered in a therapeutic dose, and followed by a molar excess of 1A7.

DETD . . . either of these applications, an amount of 1A7 polypeptide is chosen that is in sufficient molar excess over the labeled **anti-GD2** to locate and bind any **anti-GD2** that is not localized at the tumor site. The timing of administration and amount of 1A7 polypeptide will depend upon. . . the type of radioisotope used and the condition of the individual. Preferably, the molar ratio of 1A7 polypeptide to the **anti-GD2** antibody is at least about 5:1, more preferably about 25:1 to 200:1. Preferably, 1A7 polypeptide is administered 5 to 24 hours after the individual has received the **anti-GD2** antibody.

DETD The invention also includes methods of detecting the presence of an **anti-GD2** antibody bound to a tumor cell comprising the steps of treating an individual with 1A7 for a sufficient time to allow binding to the **anti-GD2** antibody, and detecting the presence of any complex formed. The intention is for the 1A7 to detect **anti-GD2** that has pre-attached to the tumor cell; or alternatively, to promote the binding of **anti-GD2** to the tumor cell by forming a polyvalent **anti-GD2**/1A7 imnmune complex. In the former instance, the **anti-GD2** is provided with a detectable label or a means by which a label can be attached. In the latter instance, either the **anti-GD2** or the 1A7 is provided with a label. Suitable labels include radiolabels such as .sup.111 In, .sup.131 I and .sup.99m Tc. The **anti-GD2** and 1A7 are administered (usually sequentially) into the subject and allowed to accumulate at the tumor site. The tumor is. . .

DETD . . . laboratories, practitioners, or private individuals. Kits embodied by this invention include those that allow someone to conduct an assay for **anti-GD2** or anti-1A7 activity, or for an 1A7 encoding sequence. An alteration in one of these components resulting, for example, from the presence of a **GD2**-associated disease or treatment directed towards it is typically compared with that in a sample from a healthy individual. The clinical. . .

DETD . . . necessarily comprises the reagent which renders the procedure specific: a reagent 1A7 antibody or polypeptide, used for detecting anti-1A7 or **anti-GD2** in the sample; or a reagent 1A7 encoding polynucleotide, used for detecting a 1A7 encoding polynucleotide in the sample. Optionally,. . .

DETD . . . by using the 14G2a mouse monoclonal antibody as immunogen for an anti-idiotype response. 14G2a binds to a unique epitope of **GD2** that is not present on other members of the ganglioside family. Since the responding animal was also a mouse, the. . .

DETD . . . against antibody recognizing isotypic or allotypic determinants; (3) Positive selection for an ability to inhibit the binding of 14G2a to **GD2**; and (4) Positive selection for an ability to induce a humoral immune response against the original tumor-associated antigen (**GD2**) in both mice and rabbits. The rest of this section provides an overview of the screening procedure, which is given. . .

DETD Subsequent screening was conducted by competition assays, in which the Ab2 was required to block the binding of 14G2a to **GD2**. This established that Ab2 recognized the paratope of 14G2a. **GD2** was provided in the form of M21/P6 cells, a human melanoma cell line expressing **GD2** at the cell surface. The nature of the assay requires the Ab2 to block the interaction between 14G2a and the. . .

DETD . . . for immunization. Sera testing positively were then assayed for ability of the Ab3 to react against the tumor-associated antigen; namely **GD2**. A preparation of **GD2** was used to coat microtiter plates, overlaid with the test serum in serial dilutions, and the Ab3

that bound was detected using labeled anti-immunoglobulin. The titer of the Ab3 binding to **GD2** defined the "quality" of Ab2, as a reflection of its capacity as an inducer of **anti-GD2**

.

DETD . . . subtyped as an IgG2aK. The specificity of 14G2a was reconfirmed by immunoperoxidase staining and flow microfluorimetry analysis using cells expressing **GD2**. Other monoclonal and myeloma mouse immunoglobulins were used as controls in various experiments herein described.

DETD . . . were directed against the paratope of 14G2a, the Ab2 were used to compete for the binding of radiolabeled 14G2a to **GD2**. This was performed conducted using M21/P6 cells, a human cancer cell line expressing **GD2** as a membrane constituent.

DETD Three Ab2, including 1A7, inhibited the binding of labeled 14G2a to the **GD2** expressing cells at amounts as low as about 25 ng. Purified control antibody demonstrated no inhibition.

DETD Since a central purpose of these experiments was to find an anti-idiotype capable of eliciting an **anti-GD2** immune response, the next screening step was to test its immunogenicity in animal models. The Ab2 would have to be not only immunogenic, but capable of raising Ab3 that cross-reacted back to the tumor antigen **GD2**.

DETD Accordingly, the monoclonal antibody that gave the strongest result in the competition experiments with the **GD2**-expressing cells was brought forward for testing in this part of the study. The other two antibodies showing specific inhibition were. . .

DETD . . . Ab2 (1A7 on the plate) by Ab3 sera. In addition, serum was checked for inhibition of .sup.125 I-14G2a binding to **GD2** positive melanoma cells (M21/P6). Also, direct binding of sera to purified **GD2**, coated onto microtiter plate, was determined by ELISA assay. Representative date from 3 BALB/c mice are shown in Table 1

DETD . . . 87 95 97

Sera

% Inhibition of Ab1 Binding to M21/P6 1:50 28 32 27

Melanoma Cells

Direct Binding to **GD2** by ELISA 1:10 0.70 0.76 0.71

(OD405 nm) PBS-BSA

Control 0.08

DETD . . . RIA of Ab1-Ab2 Binding to M21/P6 Ab3 Sera (1:10

Ab3 Sera (1:50 Binding by Ab3 Melanoma Cells by dil) to **GD2** by Immunized With dil) (CPM) Sera (1:50 dil) Ab3 Sera (1:50 dil) ELISA (OD 405 nm)

1A7-KLH + Freunds

Mouse #1. . .

DETD Results are expressed as mean value of the triplicate determinations (S.D.<10%). There was no reactivity with **GD2**-negative cell lines or unrelated gangliosides, such as GD3 and GM3.

DETD . . . 1A7+QS-21 immunized mice; however, the binding of Ab1 to melanoma cells was inhibited much more strongly and the production of **anti-GD2** antibodies (Ab1') was comparable to the other two groups. Thus, there was no additional advantage of coupling of KLH to. . .

DETD . . . (1 .times. 10.sup.6)

LS174-T Control Colon 2,973 (3.5) 2,074 (2.0) 3,944 (3.0) 2,340 (1.7)

Carcinoma Irradiated

Cells (1 .times. 10.sup.6)

GD2 (1 .mu.g) 514 (0.6) 2,121 (2.1) 2,932 (2.2) 2,520 (1.9)

GD3 (1 .mu.g) 290 (0.3) 1,346 (1.3) 1,180 (0.9) 1,285. . .

DETD . . . cells specific proliferative responses, some reactivity against control 3H1 and no reaction against control cell line LS174-T cells or ganglioside **GD2** or GD3. These data support the postulate that for T cell activation, **GD2** needs to be associated with cell surface oligopeptides. There was also no significant difference in Stimulation Index obtained with any. . .

DETD . . . of irradiated M21/P6 cells or irradiated LS174-T (control)

cells. In another experiment, mice received intradermal foot pad injection of purified **GD2** or purified GD3. Mice were observed for development of DTH response at the inoculation site at 24 hours and 48 hours. There were significant DTH responses directed at **GD2** -positive M21/P6 cells but not **GD2**-negative LS174-T cells in all three groups of immunized mice (data not shown). There was, however, no DTH reactivity directed at **GD2** or GD3 in any of the groups of immunized mice.

DETD . . . 41 37 42 35 44

Binding to M21/P6
Melanoma Cells
Direct Binding to 1:10 0.64 0.59 0.18 0.95 0.17 1.75
GD2 by ELISA (OD
450 nm)

DETD Results are expressed as the mean value of triplicate determinations (S.D.<10%). There was no reactivity with **GD2**-negative cell lines or unrelated gangliosides (GD3, GM3, etc.). The O.D. value obtained with PBS-BSA control was 0.08.

DETD KLH-coupled 1A7 plus QS-21 induced higher levels of anti-isotypic and anti-allotypic responses in all three rabbits. Ab3 and **GD2** -positive cell binding inhibition reactions were better in all three 1A7+QS-21 immunized rabbits. Two out of 3 rabbits in each group raised **anti-GD2** antibodies, and the response was better in 1A7+QS-21 immunized group as compared to 1A7-KLH +QS-21 group.

DETD . . . rabbit Ab3 sera were cytotoxic to M21/P6 and EL4 cells by in vitro ADCC assay, and the isotype of the **anti-GD2** antibodies in the rabbit sera was mostly of IgG type with trace amount of IgM. The Ab1' antibody in rabbit sera also reacted with melanoma cells but not with **GD2**-negative carcinoma cells by FACS analysis. Table 5 demonstrates representative data from immunized rabbits for PBL-transformation assay.

DETD . . . 28,040 14.08

Lymphoma Cells
(1 .times. 10.sup.6),
Irradiated
LS174-T Colon 5,196 1.07 3,131 1.57
Carcinoma Cells
(1 .times. 10.sup.6),
Irradiated
GD2 (1 .mu.g) 11,345 2.35 5,988 3.00
GD3 (1 .mu.g) 7,329 1.52 4,678 2.34
Medium 4,816 1.0 1,991 1.0

DETD . . . demonstrate that inununization of rabbits with both 1A7-KLH+QS-21 and 1A7-QS-21 induced T cell proliferation in PBL against anti-Id 1A7, irradiated **GD2**-positive M21/P6 cells and EL4 cells but not against **GD2**-negative LS174-T cells or against **GD2** and GD3. There was insignificant stimulation against normal isotype-matched control Ab2 (S.I.<3.0). Stimulation Index against various stimuli was almost identical. . .

DETD As a model more closely related to humans, we have investigated the effect of anti-Id 1A7 on the induction of **GD2**-specific humoral responses in cynomolgus monkeys (Macaca fascicularis). The normal tissue distribution of **GD2** in cynomolgus monkeys is very similar to that in human. As such, this primate model is ideal to gauge toxicities. . .

DETD To measure **anti-GD2** reactivity in the serum of immunized monkeys, purified **GD2** (250 ng/well) was absorbed into 96-well plates. After blocking wells with 1% BSA in PBS, test serum and Ab1 were. . .

DETD To determine whether 1A7 immunized monkey sera bound specifically to **GD2**-positive melanoma cells, the binding of monkey Ab3 sera to the melanoma cell line M21/P6 was tested. M21/P6 cells (2.times.10.sup.6) were. . .

DETD . . . melanoma cells but not with the antigen-negative MCF-7 breast cancer cell line. The Ab3 sera also bound specifically to purified **GD2** coated onto microtiter plates by ELISA. Control sera from preimmune monkeys or monkeys immunized with unrelated Ab2 (3H1) did not show appreciable binding to **GD2**. In parallel experiments, the

same Ab3s from monkey PRO 685 were compared on a plate coated with CEA (an unrelated. . .

DETD . . . QS-21. The reaction was developed with goat anti-human (F(ab')2 IgG-FITC-labeled antibody. In Panel B, MOLT-4 cells that do not express **GD2** were reacted with preimmune and immune monkey Ab3 sera raised against 1A7 plus QS-21. In Panel C, tumor cells (M21/P6). . . was developed with goat-anti-mouse-F(ab')2 IgG-FITC-labeled antibody. The results show that Ab3 from immune but not pre-immune sera was specific for **GD2**-bearing M21/P6 cells.

DETD FIG. 8 shows results from an experiment in which Ab3 was shown to bind directly to the **GD2** target antigen in a specific fashion. 250 ng of different gangliosides were coated into 96-well plate. After blocking, 50 .mu.g. . .

DETD Reactivity of immunized sera and purified Ab3 for **anti-GD2** antibodies against various gangliosides was also measured by immunoblotting (FIG. 10). Purified gangliosides (2 .mu.g each of GM3, GM2, GM1, GD3, **GD2** and GT1b) were spotted on strips of PVDF cellulose membrane at 1 cm intervals. After blocking with 3% BSA in. . .

DETD The results clearly demonstrate that 1A7-QS21 immunized monkey Ab3 antibody binds to the same antigen **GD2** as Ab1.

DETD The induction of Ab3 responses in monkeys did not cause any apparent side effects in animals despite the presence of **GD2** in some normal tissues. Only mild local swelling and irritation were observed at the injection site as a result of. . .

DETD Eligible patients are those having metastatic melanoma that is confirmed as bearing the **GD2** antigen. Patients must have a life expectancy greater than six months, adequate nutrition, non-pregnant, Southwest Oncology Group performance score 0,. . .

DETD . . . murine antibody is tested by sandwich RIA. Sera are also be tested for the ability to inhibit the binding of **anti-GD2** mAb to **GD2** antigen. The immune profile of patients is further assessed by testing the proliferative response of patient's lymphocytes to anti-id antibody, purified **GD2** antigen, and irradiated tumor cells and the cytotoxicity of patient's lymphocytes for **GD2**-positive HLA-matched cell lines or autologous tumor cells (where possible).

DETD . . . immune responses directed against native target antigens, patients' Ab3 sera is tested for reactivity with cell lines known to express **GD2** in a RIA, and also by FACS analysis, using anti-human IgG and IgM tracer reagents. In addition, the sera is checked for reactivity against a solubilized purified preparation of **GD2** antigen coated onto microtiter plates. The antigen-antibody reaction is detected by using .sup.125 I-labeled anti-human Ig reagents. Pre-immune sera is used as a control. Unrelated antigen is also used in the assay. Isotype of human Ab3 sera binding to **GD2** antigen is determined by ELISA using anti-human isotype specific reagents.

DETD . . . and Ab1 bind to the same antigenic determinant, inhibition of 14G2a binding to an Ag positive tumor cell line or **GD2** antigen by Ab3 sera is determined in an RIA. If Ab3 in patients' sera bind specifically to tumor cells, the. . .

DETD . . . at a number of doses, the titer of specific anti-tumor response (Ab1') in the sera by ELISA assay against purified **GD2** antigen coated plates is compared among different dose levels.

DETD If **anti-GD2** cannot be detected in patient sera positive for anti-1A7, it may be because the Ab1' are bound to patients' tumor. . . after the fourth immunization are cultured with various concentrations of 1A7 or unrelated Ab2 (10 .mu.g to 100 ng), or **GD2** antigen in a modified Mishell-Dutton culture. Culture supernatants are harvested and checked first for the production of specific human immunoglobulins. . .

DETD Whether a specific T Cell response to the tumor associated glycolipid **GD2** is generated in the 1A7 treated melanoma patients is tested by the following criteria: (1) if a T cell response is present which targets **GD2** on the tumor cells, and (2) whether this response increases with repeated immunizations. Analysis proceeds in 2 phases. The first. . . If this occurs, the next step is to determine whether these T cells, can lyse or release cytokines against autologous **GD2** bearing melanoma tumor cells or allogeneic **GD2** expressing melanoma cells sharing a single HLA antigen in common with

the autologous CTL.

DETD . . . of tumor infiltrating lymphocytes (TIL). Similar studies are run using TIL to determine if tumor biopsies become a source of **GD2** specific cells. Also, tumor biopsies provide a source of tumor cells to serve as critical autologous targets for cytotoxicity assays,. . .

DETD . . . the NK sensitive line K562, the LAK sensitive line Daudi, autologous tumor if available and other HLA matched and mismatched **GD2** bearing melanoma tumor cells. Preferably, a panel of over 40 well characterized melanoma tumor cell lines each expressing both class. . .

DETD . . . their own tumor cells using the anti-id 1A7 molecule. Studies are then done to determine if the antigen recognized is **GD2** on the tumor cells and identify the possible mechanisms of recognition.

DETD Objectives of this study include: (1) determination of an optimal dose to elicit an immune response against **GD2** in the various arms of the immune system; a T cell response being particularly desirable; (2) ideally, remission or palliation. . .

DETD The objectives of this study comprise ascertaining the effects of the 1A7 in patients who have been treated for a **GD2**-associated cancer and have no clinical manifestations of the disease. Ideally, 1A7 given at an optimal dose lessens the risk or. . .

DETD Eligible patients are those with **GD2**-positive small cell lung cancer. All of the patients must have entered a complete clinical remission following standard chemotherapy, and be. . .

DETD Blood samples are obtained monthly prior to each treatment. Serum levels of Ab3 (anti-1A7), Ab1' (**anti-Gd2**) and human anti-mouse antibody (HAMA) are measured by standard immunoassay. The specificity of these responses is confirmed by indirect immunoprecipitation. . . SDS-PAGE. Sera is also tested for the ability to inhibit the binding of labeled 1A7 to M21/P6 cells or purified **GD2**.

DETD . . . Hypaque.TM.. The peripheral blood mononuclear cells (PBMC) are removed, washed, and the lymphocyte precursor frequency is determined. Immunostaining for CD3, **CD28**, and CD45R markers is used to measure and sort cytotoxic T cells from suppressor T cells, using three-color flow cytometry.. . . is determined. Cytotoxicity assays are conducted using HLA-matched colon cancer cell lines or autologous tumor cells. Suppressor cell function of CD8+**CD28**+CD45R+cells is measured as the suppression of B cell immunoglobulin secretion.

DETD . . . cell binding competition assay is performed to investigate whether the 1A7 scFv retains the antigen mimicry shown by intact 1A7. **GD2**-positive M21/P6 cells (1.times.10.sup.5 cells/well in 50 .mu.l volume) are placed in a 96-well plate. The cells are incubated for 2. . .

DETD . . . number of different fragments, constructs, plasmids, and fusion proteins are contemplated in this invention as a second generation vaccine for **GD2**-associated tumors. Animals have been established in the examples given so far as suitable for testing whether a candidate vaccine can. . .

DETD Cheung et al. (1993, Int. J. Cancer 54:499-505) reported that murine lymphoma EL4 cells express **GD2** at high density. We first wanted to see if MAb 14G2a binds to EL4 cells. Essentially 100% of the EL4. . . effectively inhibit the binding of 125-labeled 14G2a to EL4 cells. Immunization of C57BL/6 mice with anti-Id 1A7 plus QS-21 induced **anti-GD2** antibodies which bind to EL4 cells and kill EL4 cells in in vitro ADCC assay. Also, spleen cells from immunized. . .

DETD (iii) **GD2**-KLH plus QS-21 (Antigen Vaccine)

DETD The serum levels of anti-anti-Id (Ab3) and **anti-GD2** antibodies is measured as described elsewhere in this disclosure. Typically, blood samples are obtained before vaccination and ten days after each immunization and assayed for **anti-GD2** antibodies. The time course is determined over which the immune response develops, the intensity of the immune response, the effect. . . multiple injections of vaccine (boosting), duration of the humoral response and variability of the humoral response between animals. Comparing the **anti-GD2** titers with survival of tumor challenge establishes whether there is any correlation between the level of humoral response and tumor. . . serum Ab3 is also studied by in

vitro ADCC or CMC assays. The target cells are EL4. The isotypes of **anti-GD2** antibodies in the serum of mice are determined by ELISA using isotype specific reagents.

DETD . . . placed into in vitro cultures. The splenocytes are then stimulated with either media alone, phytohemagglutinin (PHA), irradiated EL4 cells, purified **GD2**, anti-Id 1A7 or an irrelevant Ab2. Cell proliferation is measured after 5 days of culture and then stimulation for 18. . .

DETD One of the effector mechanism thought to be important for tumor protection is antigen specific CTL killing. EL4 or **GD2** specific CTL activity will be assayed to determine if the vaccines induce this type of cellular response. Splenocytes are harvested. . .

DETD . . . the time of tumor challenge and protection from tumor growth. Different types of vaccines are also compared (anti-Id protein, cells, **GD2**-KLH or DNA) for their ability to stimulate a protective immune response.

DETD Experiments are conducted to determine the immune effector arm involved in protective immunity against syngeneic **GD2** antigen bearing tumors. Adoptive transfer of immune Ab3 serum (containing Ab1') or immune T-lymphocyte subsets (CD4+ or CD8+) or NK. . .

DETD Cheresh, D. A., et al. Biosynthesis and expression of the disialoganglioside **GD2**, a relevant target antigen on small cell lung carcinoma for monoclonal antibody-mediated cytolysis. Cancer Res. 46:5412-5118, 1996.

DETD Mujoo, K., et al. Disialoganglioside **GD2** on human neuroblastoma cells. Target antigen for monoclonal antibody-mediated cytolysis and suppression of tumor growth. Cancer Res. 47:1098-1104, 1987.

DETD Cheung, N-K.V., et al. Ganglioside **GD2** specific monoclonal antibody 3F8. a Phase I study in patients with neuroblastoma and malignant melanoma. J. Clin. Oncol. 5:1430-1440, 1987.

DETD . . . R. F. and Morton, D. L. Regression of cutaneous metastatic melanoma by intralesional injection with human monoclonal antibody to ganglioside **GD2**. Proc. Natl. Acad. Sci. USA. 83:8694-8698, 1986.

DETD Saleh, M. N., et al. Phase I of the murine monoclonal **anti-GD2** antibody 14G2a in metastatic melanoma. Cancer Res. 52: 4342-4347, 1992.

DETD Cheung, N-K. Cheung, et al. Antibody response to murine **anti-GD2** monoclonal antibodies: Correlation with patient survival. Cancer Res. 54:2228-2233, 1994.

DETD Handgretinger, R., et al. A Phase I study of neuroblastoma with the anti-ganglioside **GD2** antibody 14G2a. Cancer Immunol. Immunother. 35:199-204, 1992.

DETD Cheresh, D. A., et al. Localization of the gangliosides **GD2** and G.sub.p3 in adhesion plaques and on the surface of human melanoma cells. Proc. Natl. Sci. USA, 81:5767-5771, 1984.

DETD Kaufman, H., et al. A recombinant vaccinia virus expressing human carcinoembryonic antigen (**GD2**). Int. J. Cancer, 48:900-907, 1991.

DETD Sen, G., et al. Murine Monoclonal Antibody-idiotype Antibody Breaks Tolerance and Induces Specific Antibody Response to Human Disialoganglioside **GD2** in Cynomolgus Monkeys. Abstract presented at the 9th International Congress of Immunology, San Francisco, Calif., July 23-29, A5250, p885, 1995

DETD . . . N., Stapleton, J. D., Khazaeli M. B. and LoBuglio, A. F. Generation of a human anti-idiotypic antibody that mimics the **GD2** antigen. J. Immunol. 151:33909-3398, 1993.

CLM What is claimed is:

1. Monoclonal antibody 1A7 in an amount sufficient to elicit an **anti-GD2** immunological response in a human, wherein monoclonal antibody 1A7 has the light and heavy chain variable region sequences contained in. . .

2. A sterile composition, comprising the amount of monoclonal antibody 1A7 according to claim 1 sufficient to elicit an **anti-GD2** immunological response in a human.

. . . antibody producing cell deposited under ATCC Accession No. HB-11786, or the progeny thereof, in an amount sufficient to elicit an **anti-GD2** immunological response in a human.

4. A sterile composition, comprising the amount of the purified antibody according to claim 3 sufficient to elicit an **anti-GD2** immunological response in a human.

5. A polypeptide capable of eliciting an **anti-GD2** immunological response in a mammal, comprising an imnmunoglobulin variable region containing the three light chain complementarity determining regions (CDRs) of. . .

6. A polypeptide according to claim 5, wherein the **anti-GD2** immunological response comprises production of **anti-GD2** antibody by the mammal.

7. A polypeptide according to claim 3, wherein the **anti-GD2** immunological response comprises production of **anti-GD2** reactive T cells by the mammal.

24. The polypeptide of claim 5 in an amount sufficient to elicit an **anti-GD2** immunological response in a human.

25. A sterile composition, comprising the amount of polypeptide according to claim 24 sufficient to elicit an **anti-GD2** immunological response in a human.

27. A kit for detection or quantitation of an **anti-GD2** antibody in a sample, comprising the polypeptide of claim 5 in suitable packaging.

28. A method for determining **anti-GD2** antibody in a sample, comprising the steps of: a) contacting antibody in the sample with the polypeptide of claim 5. . . correlating the absence, presence or amount of stable complex detected in step b) with the absence, presence, or amount of **anti-GD2** antibody in the sample.

29. A method for purifying **anti-GD2** antibody from a sample, comprising the steps of forming a complex between the **anti-GD2** antibody and the polypeptide of claim 5 to form an antibody-polypeptide complex, separating the complex from other components of the. . .

30. A method for detecting the presence of an **anti-GD2** antibody bound to a tumor cell, comprising contacting the tumor cell with a polypeptide according to claim 5 under conditions that permit the polypeptide to bind to the **anti-GD2** antibody, and detecting any polypeptide that has bound.

31. A method of eliciting an **anti-GD2** immune response in a human, comprising administering to the human an effective amount of the product of claim 1.

32. A method of eliciting an **anti-GD2** immune response in a mammal, comprising administering to the individual an effective amount of the polypeptide of claim 5.

=> d bib abs kwic l15 3

L15 ANSWER 3 OF 10 USPATFULL
AN 1999:124469 USPATFULL
TI Methods and compositions for targeting the vasculature of solid tumors
IN Thorpe, Philip E., Dallas, TX, United States
Burrows, Francis J., San Diego, CA, United States
PA Board of Regents, The University of Texas System, Austin, TX, United States (U.S. corporation)
PI US 5965132 19991012
AI US 1994-350212 19941205 (8)
RLI Continuation-in-part of Ser. No. US 1994-205330, filed on 2 Mar 1994 which is a continuation-in-part of Ser. No. US 1992-846349, filed on 5 Mar 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Kight, John; Assistant Examiner: Hartley, Michael G.
LREP Arnold, White & Durkee
CLMN Number of Claims: 16
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 5943
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates generally to methods and compositions for targeting the vasculature of solid tumors using immunological- and growth factor-based reagents. In particular aspects, antibodies carrying diagnostic or therapeutic agents are targeted to the vasculature of solid tumor masses through recognition of tumor vasculature-associated antigens, such as, for example, through endoglin binding, or through the specific induction of endothelial cell surface antigens on vascular endothelial cells in solid tumors.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
SUMM . . . CD14 and FcR for IgE, which will activate the release of IL-1 and TNF.alpha.; and CD16, CD2 or CD3 or **CD28**, which will activate the release of IFN.gamma. and TNF.beta., respectively.
SUMM . . . for IgE, found on Mast cells; FcR for IgG (CD16), found on NK cells; as well as CD2, CD3 or **CD28**, found on the surfaces of T cells. Of these, CD14 targeting will be the most preferred due to the relative. . .
SUMM . . . other cytokines. Thus, for the practice of this aspect of the invention, one will desire to select CD2, CD3 or **CD28** (most preferably **CD28**) as the cytokine activating antigen.
SUMM In particular embodiments, the activating antigen induced by the bispecific antibody will be CD2, CD3, CD14, CD16, FcR for IgE, **CD28** or the T-cell receptor antigen, as may be the case. However, preferably, the bispecific antibody will recognize CD14, and induce the expression of IL-1 by monocyte/macrophage cells in the tumor, or recognize **CD28** and induce the expression of IFN-.gamma. by T-cells in the tumor. Where IL-1 is the cytokine intermediate, the second antibody. . .
DETD . . . bispecific antibodies such as these is predicated in part on the fact that cross-linking antibodies recognizing CD3, CD14, CD16 and **CD28** have previously been shown to elicit cytokine production selectively upon cross-linking with the second antigen (Qian et al., 1991). In. . .
DETD . . . vascular endothelium. Alternatively, the bispecific antibody may be targeted to FcR for IgE, FcR for IgG (CD16), CD2, CD3, or **CD28**, and achieve a similar result, with the cytokine intermediate and cytokine-producing leukocyte being different or the same.
DETD . . . macrophages
CD14
Molecule-1 Molecule-110
IL-1, TNF-
mast cells FcR for IgE
(INCAM-110)
.alpha.
(Immunoglobulin

			TNF-.beta., IL-		
				helper T cells	
					CD2, CD3, **CD28**
	Family)		4		
			TNF	NK cells	FcR for IgG (CD16)
Intercellular					
	ICAM-1				
	--		IL-1, TNF.alpha.		
				monocytes	CD14
Adhesion	(Immunoglobulin				
			(Bacterial		
				macrophages	
					CD15
Molecule-1	Family)		Endotoxin)		
				mast cells	FcR for IgE
			TNF-.beta.,		
				T helper cells	
					CD2, CD3, **CD28**
			IFN.gamma.		
				NK cells	FcR for IgG (CD16)
The Agent for					
	LAM-1 MEL-14 Agent				
			Il-1, TNF.alpha.		
				monocytes	CD14
Leukocyte	Agent (Mouse)		(Bacterial		
				macrophages	
					CD14
Adhesion			Endotoxin)		
				mast cells	FcR for IgE
Molecule-1					
Major	MHC	HLA-DR	Human		
			IFN-.gamma.		
				helper T cells	
					CD2, CD3, **CD28**
histocompatibility					
	Class	HLA-DP		NK cells	FcR for IgG (CD16)
Complex	II	HLA-DQ			
Class II		I-A	Mouse	NK cells	FcR for IgG (CD16)
Antigen		I-E			

DETD . . . bispecific antibody against a solid tumor antigen that activates Thl cells in the tumor in a CsA-independent fashion, such as **CD28**. Such an antibody will trigger the release of IFN-.gamma. which, in turn, will result in the selective expression of Class. . .

DETD . . . be more suitable for the MHC Class II approach involving, e.g., the cross-linking of T-cells in the tumor through an anti-**CD28** /anti-tumor bispecific antibody, because these tumors are more likely to be infiltrated by T cells, a prerequisite. Examples of immunogenic solid. . .

DETD . . . the tumor. A bispecific (Fab'-Fab') antibody having one arm directed against a tumor antigen and the other arm directed against **CD28** should localize in the tumor and then crosslink **CD28** antigens on T cells in the tumor. Crosslinking of **CD28**, combined with a second signal (provided, for example, by IL-1 which is commonly secreted by tumor cells (Burrows et al.,. . .

DETD . . . involves the preparation of peptic F(ab'.gamma.).sub.2 fragments from the two chosen antibodies (e.g., an antitumor antibody and an anti-CD14 or anti-**CD28** antibody), followed by reduction of each to provide separate Fab'.gamma.SH fragments. The SH groups on one of the two partners. . .

DETD . . . various cytokine activating molecules is also well known in the art. For example, the preparation and use of anti-CD14 and anti-**CD28** monoclonal antibodies having the ability to induce cytokine production by leukocytes has now been described by several laboratories (reviewed in. . .

DETD . . . Int. J. Cancer, 27:775, 1981

glioblastomas
bladder & "Ca Antigen"
CA1 Ashall et al., Lancet, July 3, 1, 1982

laryngeal cancers
350-390 kD
neuroblastoma
GD2 3F8 Cheung et al., Proc. AACR, 27:318, 1986
Prostate gp48 48 kD GP
4F.sub.7 /7A.sub.10
Bhattacharya et al., Cancer Res. 44:4528,

. . .

DETD . . . to the extent that Class II disappears from the vasculature. The mice will then be injected with a bispecific (Fab'-Fab') anti-**CD28**/anti-Ly6A.2 antibody, which should localize to the tumor by virtue of its Ly6.2-binding activity. The bispecific antibody should then bind to T cells which are present in (or which subsequently infiltrate (Blanchard et al., 1988) the tumor. Crosslinking of **CD28** antigens on the T cells by multiple molecules of bispecific antibody attached to the tumor cells should activate the T cells via the CsA-resistant **CD28** pathway (Hess et al., 1991; June et al., 1987; Bjorndahl et al., 1989). Activation of T cells should not occur elsewhere because the crosslinking of **CD28** antigens which is necessary for activation (Thompson et al., 1989; Koulova et al., 1991) should not occur with soluble, non-tumor. . .

DETD An appropriate anti-mouse **CD28** antibody (Gross, et al., 1990) is that obtainable from Dr. James Allison (University of California, Calif.). Ascitic fluid from hybridoma-bearing. . .

DETD . . . by Ghetie, et al. (1988). The ability of the purified anti-Ly6A.2 antibody to bind to MM102 cells and of the anti-**CD28** antibody to bind mouse T cells will be confirmed by FACS analyses as described by Burrows et al., (1991).

DETD F(ab').sub.2 fragments of purified anti-Ly6A.2 and anti-**CD28** antibodies will be prepared by pepsin digestion, as described by Glennie et al. (1987). Purified antibodies (5-10 mg) will be. . .

DETD d) Preparation of anti-Ly6A.2/anti-**CD28** bispecific antibodies

DETD For the production of anti-Ly6A.2-anti-**CD28** bispecific antibodies, Fab' fragments of each antibody will be initially prepared as above and will be left unalkylated. Heterodimer molecules. . .

DETD e) Confirmation of cell binding-capacity of anti-Ly6A.2/anti-**CD28** bispecific-antibody

DETD . . . that the bispecific antibody is intact and is capable of binding tumor cells. The study will be repeated using a **CD28** positive mouse T cell lymphoma line (e.g., EL4) and with fluoresceinated goat anti-mouse immunoglobulin as the detecting antibody to confirm that the bispecific antibody has **CD28**-binding capacity.

DETD f) Activation of T cells by anti-Ly6A.2/anti-**CD28** bispecific antibody plus MM102 tumor cells

DETD . . . cells will be cultured (0.5 to 1.times.10.sup.5 cells/0.2 ml) in medium in the wells of 96-well plates. Various concentrations of anti-**CD28** IgG, anti-**CD28** Fab' or anti-Ly6A.2/anti-**CD28** bispecific antibody will be added together with various concentrations of one of the following costimulants: PMA, IL1 or anti-CD3 IgG.. . . .sup.3 H-thymidine (1 .mu.Ci/culture) will be added and the plates harvested 24 hours later. These studies should confirm that bivalent anti-**CD28**, but not monovalent Fab' anti-**CD28** or the bispecific antibody, stimulate T cells and that the stimulation is not CsA inhibitable.

DETD g) Confirmation that injection of anti-Ly6A.2/anti-**CD28** bispecific antibody into CsA-treated MM102 tumor-bearing mice results in induction of Class II selectively on tumor vasculature

DETD . . . in diameter, and when Class II will have disappeared from the vasculature, mice will be injected with 50-100 .mu.g of anti-Ly6A.2/anti-**CD28** bispecific antibody. Other mice will receive various control treatments, including unconjugated anti-Ly6A.2 or anti-**CD28** (Fab' and IgG) or diluent alone. Two or three days later, the mice will be sacrificed and the tumors and. . .

DETD . . . IFN-.gamma. secretion to ensue. If so, the presence of T cells will be verified by staining frozen tumor sections with anti-**CD28** and anti-CD3 antibodies. If T cells are present, again as would be anticipated from prior studies (Koulova, et al., 1991;. . . a 2nd signal might be missing. This will be checked by coadministering

an anti-Ly6A.2/anti-CD3 bispecific antibody, which together with the anti-Ly6A.2/anti-**CD28** bispecific, should provide the signalling needed for T cell activation.

DETD . . . mg/kg/d). When the tumors have grown to 1.0-1.3 cm diameter, the mice will receive an intravenous injection of 50-100 .mu.g anti-Ly6A.2/anti-**CD28** bispecific antibody (perhaps together with anti-Ly6A.2/anti-CD3 bispecific antibody if indicated by the studies in Section (h) above). Two or three. . . comparing the anti-tumor effects with those in mice which receive various control treatments, including unconjugated anti-Ly6A.2 Fab' and IgG, unconjugated anti-**CD28** Fab' and IgG, and anti-Class II immunotoxin alone.

=> d bib abs kwic l15 4

L15 ANSWER 4 OF 10 USPATFULL
AN 1999:92538 USPATFULL
TI Polynucleotides related to monoclonal antibody 1A7 and use for the treatment of melanoma and small cell carcinoma
IN Chatterjee, Malaya, Lexington, KY, United States
Foon, Kenneth A., Lexington, KY, United States
Chatterjee, Sunil K., Lexington, KY, United States
PA Board of Trustees of the University of Kentucky, Lexington, KY, United States (U.S. corporation)
PI US 5935821 19990810
AI US 1996-752844 19961121 (8)
RLI Continuation-in-part of Ser. No. US 1995-372676, filed on 17 Jan 1995, now patented, Pat. No. US 5612030 And a continuation-in-part of Ser. No. US 1996-591196, filed on 16 Jan 1996
DT Utility
EXNAM Primary Examiner: Huff, Sheela; Assistant Examiner: Reeves, Julie E.
LREP Morrison & Foerster LLP
CLMN Number of Claims: 23
ECL Exemplary Claim: 1
DRWN 27 Drawing Figure(s); 27 Drawing Page(s)
LN.CNT 4863
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to monoclonal antibody 1A7. This is an anti-idiotype produced by immunizing with an antibody specific for ganglioside **GD2**, and identifying a hybridoma secreting antibody with immunogenic potential in a multi-step screening process. Also disclosed are polynucleotide and polypeptide derivatives based on 1A7, including single chain variable region molecules and fusion proteins, and various pharmaceutical compositions. When administered to an individual, the 1A7 antibody overcomes immune tolerance and induces an immune response against **GD2**, which comprises a combination of **anti-GD2** antibody and **GD2**-specific T cells. The invention further provides methods for treating a disease associated with altered **GD2** expression, particularly melanoma, neuroblastoma, glioma, soft tissue sarcoma, and small cell carcinoma. Patients who are in remission as a result of traditional modes of cancer therapy may be treated with a composition of this invention in hopes of reducing the risk of recurrence.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB . . . present invention relates to monoclonal antibody 1A7. This is an anti-idiotype produced by immunizing with an antibody specific for ganglioside **GD2**, and identifying a hybridoma secreting antibody with immunogenic potential in a multi-step screening process. Also disclosed are polynucleotide and polypeptide. . . various pharmaceutical compositions. When administered to an individual, the 1A7 antibody overcomes immune tolerance and induces an immune response against **GD2**, which comprises a combination of **anti-GD2** antibody and **GD2**-specific T cells. The invention further provides methods for treating a disease associated with altered **GD2** expression, particularly melanoma, neuroblastoma, glioma, soft tissue sarcoma, and small cell carcinoma. Patients who are in remission as a result. . .
SUMM . . . that gangliosides may be preferable to other types of target antigens for antibody-mediated killing of certain tumor types. Gangliosides like **GD2** have simple, well-defined structures, and the level of expression is not affected by antibody binding. In vitro studies have shown that monoclonal antibodies against gangliosides like **GD2** and GD3 potentiate lymphocyte response which could potentially be directed towards tumor cells. In addition, certain gangliosides have been implicated. . .
SUMM In particular, glycosphingolipid **GD2** is expressed at high density by tumors of human neuroectodermal origin; including malignant melanoma, neuroblastoma, glioma, soft tissue sarcoma and small cell carcinoma of the lung. The **GD2** antigen is absent in most normal tissues, except for low levels in brain and peripheral nerve.

SUMM . . . the cancers for which gangliosides hold significant promise as a target antigen (Livingston (1995) Immunol. Rev. 145:147-166). Increased expression of **GD2** has been observed in a majority of malignant melanoma cells. Several murine monoclonal **anti-GD2** antibodies were reported to suppress the growth of tumors of neuroectodermal origin in athymic (nu/nu) mice or cause remission in patients with metastatic melanoma. A human-mouse chimeric **anti-GD2** antibody remissions in patients with metastatic neuroblastoma. The mechanism is thought to involve antibody dependent cellular cytotoxicity (ADCC) or complement-mediated cytotoxicity (CMC). Clinical responses have been obtained by treating with monoclonal antibodies against GM2, **GD2** and GD3. Active immunization with a ganglioside vaccine comprising GM2 produced anti-GM2 antibodies in 50/58 patients, who survived longer on. . .

SUMM If there was a simple and reliable therapeutic strategy for providing immune reactivity against **GD2**, then the clinical prospects for these types of cancers might improve.

SUMM Unfortunately, there are several reasons why **GD2** is less than ideal as a component of an active vaccine. For one thing, **GD2** is of limited supply, and is difficult to purify. Of course, because **GD2** is a ganglioside, it cannot be generated by simple recombinant techniques. Secondly, gangliosides in general, and **GD2** in particular, are poorly immunogenic. In order to render them more immunogenic in humans, it has been necessary to conjugate. . .

SUMM Similarly, the passive administration of **anti-GD2** antibodies is less than ideal as an approach to long-term care. The amount of antibody that must be provided passively. . .

SUMM How else, then, could an active immune response against **GD2** be obtained? The network hypothesis of Lindemann and Jerne suggests a way of overcoming both the natural immune tolerance against **GD2**, and the shortage of supply of **GD2**. It relies on the fact that antibodies comprise variable region epitopes that themselves may be immunogenic, leading to the generation. . .

SUMM . . . (Kanda et al., Yamamoto et al., Hastings et al.). Saleh et al. and Cheung et al. have raised anti-idiotypes against **GD2**. Other anti-idiotypes have entered early clinical trials: for example, Mittelman et al. are using an anti-idiotype related to a high. . .

SUMM . . . disclosure outlines a particular monoclonal anti-idiotype antibody, designated 1A7. This antibody has been established as being capable of eliciting an **anti-GD2** response. It has all the desirable properties that provide for escaping immune tolerance to **GD2**, and is appropriate for treating **GD2**-associated disease.

SUMM Yet another embodiment is a method of treating a **GD2**-associated disease in an individual, comprising administering monoclonal antibody 1A7, or a polynucleotide or polypeptide of this invention. The disease may. . .

SUMM A further embodiment of this invention is a kit for detection or quantitation of an **anti-GD2** antibody or a 1A7 polynucleotide in a sample, comprising monoclonal antibody 1A7 or a polynucleotide or polypeptide of this invention. . .

DRWD FIG. 6 is a bar graph depicting inhibition of binding of .sup.125 I labeled 14G2a antibody to **GD2** positive melanoma cell line M21/P6 in the presence of different concentrations of Ab1 and monkey Ab3. Parallel inhibition curves were. . .

DRWD . . . from a FACS analysis of the binding of monkey Ab3 to tumor cells. Panel A shows the staining observed of **GD2**-expressing M21/P6 cells labeled with preimmune and immune Ab3. Panel B shows the staining observed on another cell line not expressing **Gd2**. Panel C shows control staining of M21/P6 cells using the **GD2**-specific antibody 14G2a, or no antibody.

DRWD . . . hand (solid) bar denotes the binding of Ab3 from monkey PRO#685; the right hand (hatched) bar denotes control binding by **anti-GD2** antibody 14G2a. This experiment shows the antibody induced upon immunization with the anti-idiotype 1A7 is antigen specific.

DRWD FIG. 9 is a bar graph depicting inhibition of binding of .sup.125 I-labeled 14G2a antibody to purified **GD2** by 14G2a and monkey Ab3. For each triad of bars, the left hand (solid) bar denotes monkey

PRO#778; the middle. . .

DRWD . . . with monoclonal antibody 1A7, when they are stimulated in culture using either antibody 1A7 or a cancer cell line expressing **GD2**.

DRWD . . . sera of human patients treated with antibody 1A7. Upper panel shows that the Ab3 response comprises specific antibody to ganglioside **GD2** (hatched bars) but not GD3 (solid bars). Lower panel shows that the **anti-GD2** response is predominantly IgG (hatched bars) rather than IgM (solid bars).

DRWD FIG. 13 is two graphs further characterizing purified Ab3 from three human patients. The induced Ab3 inhibits the binding of **anti-GD2** to purified ganglioside **GD2** (upper panel) or a **GD2**-expressing cancer cell line (lower panel) in a dose-dependent fashion.

DETD This invention relates to the discovery of an anti-idiotype antibody that is capable of recruiting a tumor-specific response against **GD2**. The antibody is designated 1A7. The immune response elicited by 1A7 typically comprises both humoral and cellular components, and is therefore expected to be useful in palliating the clinical conditions related to **GD2**-associated tumors. The invention comprises the 1A7 antibody molecule, along with polynucleotide and polypeptide derivatives thereof, and methods for using these. . .

DETD Cancer patients are typically tolerized to various tumor associated antigens (TAA), including **GD2**. 1A7 successfully circumvents immune tolerance, and elicits an immune response against **GD2**. According to the network theory, Ab1 represents anti-tumor monoclonal antibody; Ab2 represents anti-idiotypic monoclonal antibody; and Ab3 represents anti-anti-idiotypic monoclonal. . .

DETD . . . one explanation is that the 1A7 combining site may present a region that at least partly resembles an epitope in **GD2** in the context of one or more other epitopes which render it more immunogenic. The epitope of **GD2** which may resemble that of 1A7 is identified by the Ab1 (14G2a) used to generate 1A7. As a result, 1A7 escapes the normal immune tolerance against **GD2**, and is able to elicit an **anti-GD2** response.

DETD The 1A7 antibody and derivatives thereof are useful, for example, for eliciting an **anti-GD2** immune response, for treating a **GD2**-associated disease, and as reagents for detecting the presence of **anti GD2**.

DETD . . . and treatment modalities of this invention may be brought to bear whenever it is desirable to elicit a response against **GD2**, especially in humans. Human patients with **GD2**-associated tumors, including melanoma, neuroblastoma, glioma, soft tissue sarcoma, and small cell carcinoma (including small cell lung cancer) are especially appropriate. . .

DETD "1A7" is a particular anti-idiotype antibody raised against the **anti-GD2** monoclonal antibody with the designation 14G2a. The generation and characterization of 1A7 is described in Example 1.

DETD . . . activities: ability to specifically bind monoclonal antibody 14G2a; ability to inhibit the binding of 1A7 to 14G2a or 14G2a to **GD2** in a specific manner; and an ability to elicit an immune response against **GD2**. A specific immune response may comprise antibody, B cells, T cells, and any combination thereof, and effector functions resulting therefrom.. . .

DETD . . . other biological activity ascribed to 1A7 in this disclosure, including the role of 1A7 in the amelioration or palliation of **GD2**-associated disease.

DETD . . . following properties: ability to bind monoclonal antibody 14G2a; ability to inhibit the binding of 1A7 to 14G2a or 14G2a to **GD2** in a specific manner; and an ability to elicit an immune response with a similar antigen specificity as that elicited. . .

DETD . . . reactive against the target, or any combination thereof. For purposes of this invention, the target is primarily tumor associated antigen **GD2**, but also includes any tumor associated antigen bound by 14G2a. The immunological reactivity may be desired for experimental purposes, for. . .

DETD . . . invention, an effective amount of a 1A7 polynucleotide or polypeptide is an amount that induces an immune response, particularly an **anti-GD2** response. In terms of treatment, an

effective amount is amount that is sufficient to palliate, ameliorate, stabilize, reverse or slow the progression of the **GD2** -associated disease, or otherwise reduce the pathological consequences of the disease.

DETD 1A7 was obtained by immunizing naive mice with 14G2a **anti-GD2** antibody to obtain an anti-idiotype response. 14G2a binds to a unique epitope of **GD2**. Syngeneic BALB/c mice were immunized four times with 14G2a (Ab1) and their spleen cells were fused with the non-secretory mouse. . .

DETD . . . against antibody recognizing isotypic or allotypic determinants; (3) Positive selection for an ability to inhibit the binding of 14G2a to **GD2**; and (4) Positive selection for an ability to induce a humoral immune response against the original tumor-associated antigen (**GD2**) in both mice and rabbits.

DETD . . . determinants. To determine whether the anti-14G2a were directed against the paratope of 14G2a, the binding of radiolabeled 14G2a to the **GD2**-positive cell line M21/P6 was studied in the presence of varying amounts of Ab2 hybridoma culture supernatants. With as little as. . .

DETD . . . serum samples were titered for the presence of Ab3 that bound not only to the immunizing Ab2, but also to **GD2**. The Ab2 passing all of these screening stages was designated 1A7. Further details of the method used to obtain 1A7. . .

DETD . . . 1A7 has been further characterized. The immune sera from both mice and rabbits competed with 14G2a for binding to the **GD2** -associated cell line M21/P6 and inhibited the binding of radioiodinated 14G2a to 1A7. This indicated that anti-anti-Id (Ab3) in mice and. . . epitope as Ab1. Administration of 1A7 to non-human primates (cynomolgus monkeys) also generated a specific immune response, comprising activity against **GD2** (Example 3).

DETD . . . capable of producing a detectable signal. These conjugated antibodies are useful, for example, in detection systems such as quantitation of **anti-GD2** or tumor imaging. Such labels are known in the art and include, but are not limited to, radioisotopes, enzymes, fluorescent. . .

DETD The 1A7 antibody may be used for a number of purposes. These include eliciting an antibody response to 1A7 or **GD2**, eliciting a T cell response to 1A7 or **GD2**, and treating various types of cancer. These uses are elaborated more fully in a later section.

DETD 1A7 may also be used to purify anti-1A7 (Ab3), **anti-GD2** (Ab1'), or 14G2a (Ab1). The method comprises contacting a biological sample containing the antibody with a 1A7 polypeptide, producing a. . .

DETD The invention also encompasses methods of detecting anti-1A7 or **anti-GD2** in a biological sample. **Anti-GD2** is detectable whenever (like 14G2a) it cross-reacts with 1A7. **Anti-GD2** with this activity may spontaneously arise during the course of a **GD2**-associated disease. **Anti-GD2** with this activity is especially likely in individuals who have received a course of therapy with 1A7, or a derivative. . . for monitoring antibody levels in an individual, as well as an industrial setting, in which commercial production of anti-1A7 or **anti-GD2** is desired.

DETD The assay methods entail contacting any anti-1A7 or **anti-GD2** target antibody in the sample with a 1A7 antibody or polypeptide under conditions suitable to allow the formation of a. . .

DETD Antibody assays may be conducted entirely in fluid phase. For example, **anti-GD2** may be mixed with labeled 1A7. Alternatively, the **anti-GD2** in the sample may be used to compete with a labeled **anti-GD2** for binding sites on 1A7. Generally, bound and unbound label is separated to quantitate the percent bound. Suitable separation methods. . .

DETD . . . anti-immunoglobulin. In this assay, the amount of label associated with the solid phase is inversely related to the amount of **anti-GD2** in the sample.

DETD . . . target antibody is captured by 1A7 attached directly or through a secondary reagent to a solid phase. After washing, the **anti-GD2** is detected using anti-immunoglobulin of the appropriate species, or a second 1A7 antibody, to which a label is directly or. . . In this type of assay, the amount of label associated with the solid

phase correlates positively with the amount of **anti-GD2** in the sample. Other methods of measuring specific antibody are known in the art, and may be adapted to measure anti-1A7 or **anti-GD2** by using 1A7 as the specific reagent.

DETD 1A7 may also be used to measure the level of cellular anti-1A7 or **anti-GD2** activity. In one example, 1A7 is used to identify **anti-GD2** expressing cells in a cell suspension, perhaps B or T lymphocytes expressing a receptor that binds 1A7. 1A7 may be. . . or protein A. Suitable labels for this purpose include radiolabels and fluorescent labels. The use of fluorescent labels also allows **anti-GD2** cells to be separated from non-specific cells in a fluorescence-activated cell sorter. In a second example, **anti-GD2** expressing cells are detected in a tissue section. Typically, the tissue is fixed and embedded in a suitable medium, overlaid. . .

DETD . . . polypeptides encoded thereby. These functionally equivalent variants, derivatives, and fragments display the ability to induce an immune response, preferably an **anti-GD2** immune response. For instance, changes in a DNA sequence that do not change the encoded amino acid sequence, as well. . .

DETD . . . altering cells in vivo. The purpose may include (but is not limited to) eliciting an antibody response to 1A7 or **GD2**, eliciting a T cell response to 1A7 or **GD2**, and treating various types of cancer. These uses are elaborated more fully in a later section.

DETD . . . pair of light and heavy chains is from 1A7. In one example, each light-heavy chain pair binds different epitopes of **GD2**. Such hybrids may also be formed using chimeric heavy or light chains.

DETD . . . Constructs wherein the 1A7 polypeptide is linked directly to particle-forming protein coding sequences produce hybrids which are immunogenic for an **anti-GD2** response. The vectors also comprise immunogenic HBV epitopes; for example, the pre-S peptide and stimulate a response against HBV. Such. . .

DETD . . . are conjugated to a carrier molecule. This is desirable for a 1A7 peptide that comprises a suitable epitope for eliciting **anti-GD2**, but is too small to be immunogenic. Any conjugation method known in the art may be used. Any carrier can. . .

DETD . . . inhibit the binding between 14G2a and intact 1A7, or for its ability to specifically inhibit the binding between 14G2a and **GD2**. Alternatively, a 1A7 polypeptide can be tested for its ability to elicit an immune response, preferably an **anti-GD2** response. 1A7 polypeptides can also be tested for their ability to palliate or ameliorate **GD2**-associated disease, such as **GD2**-associated tumors. It is understood that only one of these properties need be present in order for a polypeptide to come. . .

DETD . . . the putative 1A7 polypeptide is titered for its ability to decrease the binding of 1A7 to 14G2a, or 14G2a to **GD2**. Either of the binding pairs in the reaction to be inhibited is labeled, while the other is typically insolubilized in order to facilitate washing. **GD2**, if it is used, may be provided as the purified ganglioside, or as a **GD2**-expressing cell line, like M21/P6. The 1A7 polypeptide is typically mixed with the labeled component, and then the mixture is combined. . .

DETD Preferred uses of these compounds include eliciting an antibody response to 1A7 or more preferably **GD2**, eliciting a T cell response to 1A7 or more preferably **GD2**, and treating various types of **GD2**-associated cancer. These uses are elaborated more fully in a later section.

DETD . . . 1A7 either alone or in combination. Such pharmaceutical compositions and vaccines are useful for eliciting an immune response and treating **GD2**-associated diseases, either alone or in conjunction with other forms of therapy, such as chemotherapy or radiotherapy.

DETD . . . response. Since the objective is typically to identify compositions useful in cancer therapy, the samples are preferably measured for an **anti-GD2** response, as manifest in direct or inhibition type experiments.

DETD Presence of anti-1A7 (Ab3) and **anti-GD2** (Ab1') activity in a humoral response is preferably determined after first

pre-incubating sera with autologous immunoglobulin or adsorbing on a. . . Results from this assay are compared to those obtained before administration of the 1A7 polypeptide (Example 1). Alternatively, binding to **GD2** positive cells, such as M21/P6 cells, can be tested using immune flow cytometry. In a third example, the specificity of Ab3 is determined by Western blot. **GD2** is separated by SDS-PAGE and blotted to a nitrocellulose filter. The filter is then incubated with sera containing Ab3, and the reaction developed by a suitably labeled anti-immunoglobulin. If the Ab3 binds to **GD2**, a band at the appropriate molecular weight should appear.

DETD . . . that Ab3 and 14G2a contain at least some similar binding determinants. Competition of Ab3 with the binding of 14G2a to **GD2** may also be measured.

DETD . . . its ability to elicit an antibody that is cytotoxic. For determination of complement mediated cytotoxicity (CMC), M21/P6 target cells expressing **GD2** are labeled with .sup.51 Cr. Labeling may be accomplished by incubating about 10.sup.6 cells with approximately 200 .mu.Ci Na.sub.2 SO.sub.4. . .

DETD Another way of characterizing a composition of this invention is by testing its ability to elicit an **anti-GD2** antibody that participates in an ADCC response (Cheresh et al. (1986) Cancer Research 46:5112-5118). In this assay, cultured human M21/P6 cells (which express **GD2** in their surface) are labeled with .sup.51 Cr and are used as target cells. Normal human peripheral blood mononuclear cells. . .

DETD . . . (Kantor et al. (1992) J. Natl. Cancer Inst. 84:1084-1091). An example of a .sup.51 Cr release assay is the following. **GD2** -positive tumor cells (typically 1-2.times.10.sup.6 cells) are radiolabeled as target cells with about 200 .mu.Ci of Na.sub.2 .sup.51 CrO.sub.4 (Amersham Corp.,. . .

DETD . . . way of characterizing a 1A7 polypeptide is testing its ability to ameliorate, delay the progression or reduce the extent of **GD2** -associated disease, as outlined in the following section.

DETD . . . may be used for administration to individuals. They may be administered for experimental purposes, or to obtain a source of **anti-GD2**.

DETD Compositions of this invention are particularly suitable for administration to human individuals with a **GD2**-associated disease. A **GD2** associated disease is one in which expression of the **GD2** ganglioside is altered at the affected tissue site, usually an elevation in cell-surface expression. Relevant diseases are those in which an active immune response against **GD2** would confer a clinical benefit. Especially relevant are **GD2** -associated cancers; particularly melanoma, neuroblastoma, glioma, sarcoma, and small cell lung cancer.

DETD . . . this invention may be used to elicit an immune response. This includes an anti-1A7 specific response, and more preferably an **anti-GD2** response. The desired response may be a specific antibody response; a specific T helper-inducer repines, or a specific cytotoxic T. . .

DETD Also included in this invention are methods for treating **GD2** -associated disease, such as a tumor expressing **GD2**. The method comprises administering an amount of a pharmaceutical composition effective to achieve the desired effect, be it palliation of. . .

DETD For treatment of a **GD2**-associated disease in vivo, the amount of a pharmaceutical composition administered is an amount effective in producing the desired effect. An. . .

DETD Suitable subjects include those who are suspected of being at risk of a pathological effect of any **GD2**-associated condition are suitable for treatment with the pharmaceutical compositions of this invention. Those with a history of a **GD2**-associated cancer are especially suitable.

DETD . . . insufficient to identify this population). A pharmaceutical composition embodied in this invention is administered to these patients to elicit an **anti-GD2** response, with the objective of palliating their condition. Ideally, reduction in tumor mass occurs as a result, but any clinical. . .

DETD . . . subjects is known in the art as the "adjuvant group". These are individuals who have had a history of a **GD2**-associated cancer, but have been responsive to another mode of therapy. The prior therapy

may have included (but is not restricted. . .
DETD . . . or after the initial treatment. These features are known in the clinical arts, and are suitably defined for each different **GD2**-associated cancer. Features typical of high risk subgroups are those in which the tumor has invaded neighboring tissues, or who show. . .
DETD . . . invention is administered to patients in the adjuvant group, or in either of these subgroups, in order to elicit an **anti-GD2** response. Ideally, the composition delays recurrence of the cancer, or even better, reduces the risk of recurrence (i.e., improves the. . .
DETD . . . treatment of cells ex vivo. This may be desirable for experimental purposes, or for treatment of an individual with a **GD2**-associated disease. In one example, the 1A7 antibody, or a polynucleotide or polypeptide derivative are administered to a culture of cells,. . .
DETD . . . 1A7 antibodies and polypeptide derivatives to remove a label (particularly a radiolabel) from an individual who has received a labeled **anti-GD2** antibody (such as 14G2a) in the course of radioscintigraphy or radiotherapy. Effective imaging using radiolabeled antibodies is hampered due to. . . 1A7 antibody or a polypeptide derivative is administered to the individual at a specified time after administration of the labeled **anti-GD2**. The intention is for the 1A7 polypeptide to complex with **anti-GD2** at sites other than the tumor, such as in the circulation and interstitial spaces, and thereby promote its clearance. As. . . is desirable to reduce collateral exposure of unaffected tissue. This invention thus includes methods of treatment in which a radiolabeled **anti-GD2** antibody is administered in a therapeutic dose, and followed by a molar excess of 1A7.
DETD . . . either of these applications, an amount of 1A7 polypeptide is chosen that is in sufficient molar excess over the labeled **anti-GD2** to locate and bind any **anti-GD2** that is not localized at the tumor site. The timing of administration and amount of 1A7 polypeptide will depend upon. . . the type of radioisotope used and the condition of the individual. Preferably, the molar ratio of 1A7 polypeptide to the **anti-GD2** antibody is at least about 5:1, more preferably about 25:1 to 200:1. Preferably, 1A7 polypeptide is administered 5 to 24 hours after the individual has received the **anti-GD2** antibody.
DETD The invention also includes methods of detecting the presence of an **anti-GD2** antibody bound to a tumor cell comprising the steps of treating an individual with 1A7 for a sufficient time to allow binding to the **anti-GD2** antibody, and detecting the presence of any complex formed. The intention is for the 1A7 to detect **anti-GD2** that has pre-attached to the tumor cell; or alternatively, to promote the binding of **anti-GD2** to the tumor cell by forming a polyvalent **anti-GD2**/1A7 immune complex. In the former instance, the **anti-GD2** is provided with a detectable label or a means by which a label can be attached. In the latter instance, either the **anti-GD2** or the 1A7 is provided with a label. Suitable labels include radiolabels such as .sup.111 In, .sup.131 I and .sup.99m Tc. The **anti-GD2** and 1A7 are administered (usually sequentially) into the subject and allowed to accumulate at the tumor site. The tumor is. . .
DETD . . . laboratories, practitioners, or private individuals. Kits embodied by this invention include those that allow someone to conduct an assay for **anti-GD2** or anti-1A7 activity, or for an 1A7 encoding sequence. An alteration in one of these components resulting, for example, from the presence of a **GD2**-associated disease or treatment directed towards it is typically compared with that in a sample from a healthy individual. The clinical. . .
DETD . . . necessarily comprises the reagent which renders the procedure specific: a reagent 1A7 antibody or polypeptide, used for detecting anti-1A7 or **anti-GD2** in the sample; or a reagent 1A7 encoding polynucleotide, used for detecting a 1A7 encoding polynucleotide in the sample. Optionally,. . .
DETD . . . by using the 14G2a mouse monoclonal antibody as immunogen for an anti-idiotype response. 14G2a binds to a unique epitope of **GD2** that is not present on other members of the ganglioside family. Since the responding animal was also a mouse, the. . .

DETD . . . against antibody recognizing isotypic or allotypic determinants; (3) Positive selection for an ability to inhibit the binding of 14G2a to **GD2**; and (4) Positive selection for an ability to induce a humoral immune response against the original tumor-associated antigen (**GD2**) in both mice and rabbits. The rest of this section provides an overview of the screening procedure, which is given. . .

DETD Subsequent screening was conducted by competition assays, in which the Ab2 was required to block the binding of 14G2a to **GD2**. This established that Ab2 recognized the paratope of 14G2a. **GD2** was provided in the form of M21/P6 cells, a human melanoma cell line expressing **GD2** at the cell surface. The nature of the assay requires the Ab2 to block the interaction between 14G2a and the. . .

DETD . . . for immunization. Sera testing positively were then assayed for ability of the Ab3 to react against the tumor-associated antigen; namely **GD2**. A preparation of **GD2** was used to coat microtiter plates, overlaid with the test serum in serial dilutions, and the Ab3 that bound was detected using labeled anti-immunoglobulin. The titer of the Ab3 binding to **GD2** defined the "quality" of Ab2, as a reflection of its capacity as an inducer of **anti-GD2**

.

DETD . . . subtyped as an IgG2a.kappa.. The specificity of 14G2a was reconfirmed by immunoperoxidase staining and flow microfluorimetry analysis using cells expressing **GD2**. Other monoclonal and myeloma mouse immunoglobulins were used as controls in various experiments herein described.

DETD . . . were directed against the paratope of 14G2a, the Ab2 were used to compete for the binding of radiolabeled 14G2a to **GD2**. This was performed conducted using M21/P6 cells, a human cancer cell line expressing **GD2** as a membrane constituent

DETD Three Ab2, including 1A7, inhibited the binding of labeled 14G2a to the **GD2** expressing cells at amounts as low as about 25 ng. Purified control antibody demonstrated no inhibition.

DETD Since a central purpose of these experiments was to find an anti-idiotype capable of eliciting an **anti-GD2** immune response, the next screening step was to test its immunogenicity in animal models. The Ab2 would have to be not only immunogenic, but capable of raising Ab3 that cross-reacted back to the tumor antigen **GD2**.

DETD Accordingly, the monoclonal antibody that gave the strongest result in the competition experiments with the **GD2**-expressing cells was brought forward for testing in this part of the study. The other two antibodies showing specific inhibition were. . .

DETD . . . Ab2 (1A7 on the plate) by Ab3 sera. In addition, serum was checked for inhibition of .sup.125 I-14G2a binding to **GD2** positive melanoma cells (M21/P6). Also, direct binding of sera to purified **GD2**, coated onto microtiter plate, was determined by ELISA assay. Representative date from 3 BALB/c mice are shown in Table 1

DETD . . . Binding by Ab3

Sera	1:50	87	95	97
% Inhibition of Ab1 Binding to M21/P6	1:50	28	32	27
Melanoma Cells				
Direct Binding to **GD2** by ELISA	1:10	0.70	0.76	0.71
(OD405 nm)	PBS-BSA			
	Control	0.08		

DETD There was no reactivity with **GD2** negative cell lines or unrelated gangliosides such as GD3 and GM3. Results are expressed as mean value of triplicate determinations. . .

DETD . . . Sandwich RIA of

Ab3 Sera (1:50 dilution)	Ab1-Ab2 Binding to M21/P6 Melanoma Cells by Ab3 Sera (1:10 dilution) to **GD2**
Binding by Ab3	

Sera Ab3 Sera by ELISA
Immunized with
cpm (1:50 dilution)
(1:50 dilution)
(OD 405 nm)

1A7-KLH + Freunds
Mouse #1 4,729. . .
DETD Results are expressed as mean value of the triplicate determinations (S.D.<10%). There was no reactivity with **GD2**-negative cell lines or unrelated gangliosides, such as GD3 and GM3.
DETD . . . 1A7+QS-21 immunized mice; however, the binding of Ab1 to melanoma cells was inhibited much more strongly and the production of **anti-GD2** antibodies (Ab1') was comparable to the other two groups. Thus, there was no additional advantage of coupling of KLH to. . .
DETD . . . (13.3)
12,999 (9.8)
Irradiated Cells (1 .times. 10.sup.6)
LS174-T Control Colon
2,973 (3.5)
2,074 (2.0)
3,944 (3.0)
2,340 (1.7)
Carcinoma Irradiated
Cells (1 .times. 10.sup.6)
GD2 (1 .mu.g)
514 (0.6)
2,121 (2.1)
2,932 (2.2)
2,520 (1.9)
GD3 (1 .mu.g)
290 (0.3)
1,346 (1.3)
1,180 (0.9)
1,285 (0.9)
Medium. . .
DETD . . . cells specific proliferative responses, some reactivity against control 3H1 and no reaction against control cell line LS174-T cells or ganglioside **GD2** or GD3. These data support the postulate that for T cell activation, **GD2** needs to be associated with cell surface oligopeptides. There was also no significant difference in Stimulation Index obtained with any. . .
DETD . . . of irradiated M21/P6 cells or irradiated LS174-T (control) cells. In another experiment, mice received intradermal foot pad injection of purified **GD2** or purified GD3. Mice were observed for development of DTH response at the inoculation site at 24 hours and 48 hours. There were significant DTH responses directed at **GD2** -positive M21/P6 cells but not **GD2**-negative LS174-T cells in all three groups of immunized mice (data not shown). There was, however, no DTH reactivity directed at **GD2** or GD3 in any of the groups of immunized mice.
DETD . . . of Ab1
1:100
33 41 37 42 35 44
Binding to M21/P6
Melanoma Cells
Direct Binding to
1:10
0.64
0.59
0.18
0.95
0.17
1.75
GD2 by ELISA (OD
450 nm)

DETD Results are expressed as the mean value of triplicate determinations (S.D.<10%). There was no reactivity with **GD2**-negative cell

lines or the gangliosides GD3 or GM3. The O.D. value obtained with PBS-BSA control was 0.08.

DETD KLH-coupled 1A7 plus QS-21 induced higher levels of anti-isotypic and anti-allotypic responses in all three rabbits. Ab3 and **GD2** -positive cell binding inhibition reactions were better in all three 1A7+QS-21 immunized rabbits. Two out of 3 rabbits in each group raised **anti-GD2** antibodies, and the response was better in 1A7+QS-21 immunized group as compared to 1A7-KLH+QS-21 group.

DETD . . . responses in both mice and rabbits. There was no additional advantage of coupling 1A7 to KLH. The isotype of the **anti-GD2** antibodies in the rabbit sera was mostly of IgG type with trace amount of IgM. The Ab1' antibody in rabbit sera also reacted with melanoma cells but not with **GD2**-negative carcinoma cells by FACS analysis.

DETD . . . 28,845

14.48

Melanoma Cells
(1 .times. 10.sup.6),
Irradiated
EL4 Murine

44,619

9.26 28,040

14.08

Lymphoma Cells
(1 .times. 10.sup.6),
Irradiated
LS174-T Colon

5,196

1.07 3,131

1.57

Carcinoma Cells
(1 .times. 10.sup.6),
Irradiated
GD2 (1 .mu.g)

11,345

2.35 5,988

3.00

GD3 (1 .mu.g)

7,329

1.52 4,678

2.34

Medium 4,816

1.0 1,991

1.0

DETD . . . demonstrate that immunization of rabbits with both 1A7-KLH+QS-21 and 1A7-QS-21 induced T cell proliferation in PBL against anti-Id 1A7, irradiated **GD2**-positive M21/P6 cells and EL4 cells but not against **GD2**-negative LS174-T cells or against **GD2** and GD3. There was insignificant stimulation against normal isotype-matched control Ab2 (S.I.<3.0). Stimulation Index against various stimuli was almost identical. . .

DETD . . . with an intradermal inoculum of purified gangliosides. Slight erythema and induration was observed as a result of challenge with either **GD2** or GD3. In a separate experiment, immunized rabbits were challenged with an intradermal inoculum of 1.times.10.sup.6 cells inactivated by irradiation at 12,000 rads. In a rabbit immunized with 1A7+QS-21 and challenged with M21/P6 cells (a **GD2**-expressing line), the induration was 13.times.12 mm at 24 h and 18.times.13 mm at 48 h. In a rabbit immunized with. . . was 18.times.16 mm at 24 h and 24.times.16 mm at 48 h. In contrast, when challenged with LS174-T cells (a **GD2**-negative line), the induration was 4.times.4 mm and 5.times.3 mm respectively at 24 h, and negligible at 48 h.

DETD As a model more closely related to humans, the effect of anti-Id 1A7 on the induction of **GD2**-specific humoral responses was investigated in cynomolgus monkeys (Macaca fascicularis). The normal tissue distribution of **GD2** in cynomolgus monkeys is very similar to that in human. As such, this primate model is ideal to gauge toxicities. . .

DETD To measure **anti-GD2** reactivity in the serum of

immunized monkeys, purified **GD2** (250 ng/well) was absorbed into 96-well plates. After blocking wells with 1% BSA in PBS, test serum and Ab1 were. . .

DETD To determine whether 1A7 immunized monkey sera bound specifically to **GD2** positive melanoma cells, the binding of monkey Ab3 sera to the melanoma cell line M21/P6 was tested. M21/P6 cells (2.times.10.sup.6). . .

DETD . . . melanoma cells but not with the antigen-negative MCF-7 breast cancer cell line. The Ab3 sera also bound specifically to purified **GD2** coated onto microtiter plates by ELISA. Control sera from preimmune monkeys or monkeys immunized with unrelated Ab2 (3H1) did not show appreciable binding to **GD2**. In parallel experiments, the same Ab3s from monkey PRO 685 were compared on a plate coated with CEA (an unrelated. . .

DETD . . . QS-21. The reaction was developed with goat anti-human F(ab').sub.2 IgG-FITC-labeled antibody. In Panel B, MOLT-4 cells that do not express **GD2** were reacted with preimmune and immune monkey Ab3 sera raised against 1A7 plus QS-21. In Panel C, tumor cells (M21/P6). . . was developed with goat-anti-mouse-F(ab').sub.2 IgG-FITC-labeled antibody. The results show that Ab3 from immune but not pre-immune sera was specific for **GD2**-bearing M21/P6 cells.

DETD FIG. 8 shows results from an experiment in which Ab3 was shown to bind directly to the **GD2** target antigen in a specific fashion. 250 ng of different gangliosides were coated into 96-well plate. After blocking, 50 .mu.g. . .

DETD Reactivity of immunized sera and purified Ab3 for **anti-GD2** antibodies against various gangliosides was also measured by immunoblotting (FIG. 10). Purified gangliosides (2 .mu.g each of GM3, GM2, GM1, GD3, **GD2** and GT1b) were spotted on strips of PVDF cellulose membrane at 1 cm intervals. After blocking with 3% BSA in. . .

DETD The results clearly demonstrate that 1A7-QS21 immunized monkey Ab3 antibody binds to the same antigen **GD2** as Ab1.

DETD . . . hatching, 2 .mu.g antibody 1A7; Open bars, 2 .mu.g of an unrelated murine antiidiotype; Filled bars, radiation-killed M21/P6 cells (a **GD2**-expressing cancer line); Shaded bars, radiation-killed LS174-T cells (a **GD2**-negative cancer line); Light hatching, medium control. The reactivity indicated by the open bars likely represents a cellular response to non-idiotypic. . . when stimulated with 1A7 is consistent with a cellular response to the idiotypic components of 1A7. The ability of a **GD2**-expressing cell line but not a **GD2**-negative cell line to induce proliferation suggests that the cellular response comprises **anti-GD2** activity.

DETD The induction of Ab3 responses in monkeys did not cause any apparent side effects in animals despite the presence of **GD2** in some normal tissues. Only mild local swelling and irritation were observed at the injection site as a result of. . .

DETD Eligible patients are those having metastatic melanoma that is confirmed as bearing the **GD2** antigen. Patients must have a life expectancy greater than six months, adequate nutrition, non-pregnant, Southwest Oncology Group performance score 0,. . .

DETD . . . murine antibody is tested by sandwich RIA. Sera are also be tested for the ability to inhibit the binding of **anti-GD2** mAb to **GD2** antigen. The immune profile of patients is further assessed by testing the proliferative response of patient's lymphocytes to anti-id antibody, purified **GD2** antigen, and irradiated tumor cells and the cytotoxicity of patient's lymphocytes for **GD2**-positive HLA-matched cell lines or autologous tumor cells (where possible).

DETD . . . immune responses directed against native target antigens, patients' Ab3 sera is tested for reactivity with cell lines known to express **GD2** in a RIA, and also by FACS analysis, using anti-human IgG and IgM tracer reagents. In addition, the sera is checked for reactivity against a solubilized purified preparation of **GD2** antigen coated onto microtiter plates. The antigen-antibody reaction is detected by using .sup.125 I-labeled anti-human Ig reagents. Pre-immune sera is used as a control. Unrelated antigen is also used in the assay. Isotype of human Ab3 sera binding to **GD2** antigen is determined by ELISA using anti-human isotype specific reagents.

DETD . . . and Ab1 bind to the same antigenic determinant, inhibition of 14G2a binding to an Ag positive tumor cell line or **GD2** antigen by Ab3 sera is determined in an RIA. If Ab3 in patients' sera bind specifically to tumor cells, the. . .

DETD . . . at a number of doses, the titer of specific anti-tumor response (Ab1') in the sera by ELISA assay against purified **GD2** antigen coated plates is compared among different dose levels.

DETD If the serum of a particular patient tests positive for anti-1A7 (Ab3) but negative for **anti-GD2** (Ab1'), it may be because the **anti-GD2** is bound to the patent's tumor cells. The production of **anti-GD2** is optionally demonstrated by stimulating the patients' PBMC in culture with 1A7 and then measuring **anti-GD2** activity in the supernatant.

DETD Whether a specific T Cell response to the tumor associated glycolipid **GD2** is generated in the 1A7 treated melanoma patients is tested by the following criteria: (1) if a T cell response is present which targets **GD2** on the tumor cells, and (2) whether this response increases with repeated immunizations. Analysis proceeds in 2 phases. The first. . . If this occurs, the next step is to determine whether these T cells, can lyse or release cytokines against autologous **GD2** bearing melanoma tumor cells or allogeneic **GD2** expressing melanoma cells sharing a single HLA antigen in common with the autologous CTL.

DETD . . . of tumor infiltrating lymphocytes (TIL). Similar studies are run using TIL to determine if tumor biopsies become a source of **GD2** specific cells. Also, tumor biopsies provide a source of tumor cells to serve as critical autologous targets for cytotoxicity assays,. . .

DETD . . . the NK sensitive line K562, the LAK sensitive line Daudi, autologous tumor if available and other HLA matched and mismatched **GD2** bearing melanoma tumor cells. Preferably, a panel of over 40 well characterized melanoma tumor cell lines each expressing both class. . .

DETD . . . their own tumor cells using the anti-id 1A7 molecule. Studies are then done to determine if the antigen recognized is **GD2** on the tumor cells and identify the possible mechanisms of recognition.

DETD Objectives of this study include: (1) determination of an optimal dose to elicit an immune response against **GD2** in the various arms of the immune system; a T cell response being particularly desirable; (2) ideally, remission or palliation. . .

DETD The affinity and specificity of the response to **GD2** was further confirmed by using the affinity purified Ab3 in several of the assay systems described earlier. Results are shown. . .

DETD . . . on Ab3 affinity purified from three different patients. In the upper panel, an assay plate has been coated with ganglioside **GD2** (hatched bars) or GD3 (solid bars), overlaid with purified Ab3, and then developed with alkaline phosphatase labeled anti-immunoglobulin. The results show that each patient's response comprises the production of **anti-GD2** antibody (Ab1'). In the lower panel, the plate was coated with **GD2**, overlaid with purified Ab3, and then developed with isotype-specific anti-immunoglobulin reagents. The **anti-GD2** response is apparently a mature response comprising more IgG (hatched bars) than IgM (solid bars).

DETD . . . experiments conducted using purified Ab3 from three different patients. In the upper panel, an assay plate was coated with ganglioside **GD2**, and varying amounts of purified Ab3 were tested for the ability to inhibit the binding of radiolabeled 14G2a (Ab1). Diamonds:. . . panel, varying amounts of purified Ab3 were tested for their ability to inhibit the binding of radiolabeled 14G2a to the **GD2** -expressing murine lymphoma cell line EL4. The results indicate that the Ab3 induced by administration of 1A7 competes for binding to **GD2** both in plate-binding assays and when presented on cancer cells.

DETD The objectives of this study comprise ascertaining the effects of the 1A7 in patients who have been treated for a **GD2**-associated cancer and have no clinical manifestations of the disease. Ideally, 1A7 given at an optimal dose lessens the risk or. . .

DETD Eligible patients are those with **GD2**-positive small cell lung cancer. All of the patients must have entered a complete clinical remission following standard chemotherapy, and be. . .

DETD Blood samples are obtained monthly prior to each treatment. Serum levels

of Ab3 (anti-1A7), Ab1' (**anti-Gd2**) and human anti-mouse antibody (HAMA) are measured by standard immunoassay. The specificity of these responses is confirmed by indirect immunoprecipitation. . . SDS-PAGE. Sera is also tested for the ability to inhibit the binding of labeled 1A7 to M21/P6 cells or purified **GD2**.

DETD . . . FICOLL-HYPAQUE.RTM.. The peripheral blood mononuclear cells (PBMC) are removed, washed, and the lymphocyte precursor frequency is determined. Immunostaining for CD3, **CD28**, and CD45R markers is used to measure and sort cytotoxic T cells from suppressor T cells, using three-color flow cytometry.. . . is determined. Cytotoxicity assays are conducted using HLA-matched colon cancer cell lines or autologous tumor cells. Suppressor cell function of CD8+**CD28**+CD45R+cells is measured as the suppression of B cell immunoglobulin secretion.

DETD . . . cell binding competition assay is performed to investigate whether the 1A7 scFv retains the antigen mimicry shown by intact 1A7. **GD2**-positive M21/P6 cells (1.times.10.sup.5 cells/well in 50 .mu.l volume) are placed in a 96-well plate. The cells are incubated for 2. . .

DETD . . . with a pH .about.2.5 glycine buffer. Further investigation of the specificity of the scFv is conducted using the affinity-purified protein. **GD2**-positive M21/P6 cells (1.times.10.sup.5 cells/well in 50 .mu.l volume) are placed in a 96-well plate. The cells are incubated for 2. . .

DETD Mice are bled 7 days after each immunization for determination of anti-1A7 and **anti-GD2** activity as described elsewhere in this disclosure. Three mice are sacrificed from each group for isolation of spleens for the. . .

DETD . . . number of different fragments, constructs, plasmids, and fusion proteins are contemplated in this invention as a second generation vaccine for **GD2**-associated tumors. Animals have been established in the examples given so far as suitable for testing whether a candidate vaccine can. . .

DETD Cheung et al. (1993, Int. J. Cancer 54:499-505) reported that murine lymphoma EL4 cells express **GD2** at high density. MAb 14G2a was tested for binding to EL4 cells. Essentially 100% of the EL4 cells (a gift. . . effectively inhibit the binding of 125-labeled 14G2a to EL4 cells. Immunization of C57BL/6 mice with anti-Id 1A7 plus QS-21 induced **anti-GD2** antibodies which bind to EL4 cells and kill EL4 cells in in vitro ADCC assay. Also, spleen cells from immunized. . .

DETD (iii) **GD2**-KLH plus QS-21 (Antigen Vaccine)

DETD The serum levels of anti-anti-Id (Ab3) and **anti-GD2** antibodies is measured as described elsewhere in this disclosure. Typically, blood samples are obtained before vaccination and ten days after each immunization and assayed for **anti-GD2** antibodies. The time course is determined over which the immune response develops, the intensity of the immune response, the effect. . . multiple injections of vaccine (boosting), duration of the humoral response and variability of the humoral response between animals. Comparing the **anti-GD2** titers with survival of tumor challenge establishes whether there is any correlation between the level of humoral response and tumor. . . serum Ab3 is also studied by in vitro ADCC or CMC assays. The target cells are EL4. The isotypes of **anti-GD2** antibodies in the serum of mice are determined by ELISA using isotype specific reagents.

DETD . . . placed into in vitro cultures. The splenocytes are then stimulated with either media alone, phytohemagglutinin (PHA), irradiated EL4 cells, purified **GD2**, anti-Id 1A7 or an irrelevant Ab2. Cell proliferation is measured after 5 days of culture and then stimulation for 18. . .

DETD One of the effector mechanism thought to be important for tumor protection is antigen specific CTL killing. EL4 or **GD2** specific CTL activity will be assayed to determine if the vaccines induce this type of cellular response. Splenocytes are harvested. . .

DETD . . . the time of tumor challenge and protection from tumor growth. Different types of vaccines are also compared (anti-Id protein, cells, **GD2**-KLH or DNA) for their ability to stimulate a protective immune response.

DETD Experiments are conducted to determine the immune effector arm involved in protective immunity against syngeneic **GD2** antigen bearing tumors. Adoptive transfer of immune Ab3 serum (containing Ab1') or immune T-lymphocyte subsets (CD4+ or CD8+) or NK. . .

DETD Cheresh, D. A., et al. Biosynthesis and expression of the disialoganglioside **GD2**, a relevant target antigen on small cell lung carcinoma for monoclonal antibody-mediated cytolysis. Cancer Res. 46:5412-5118, 1996.

DETD Mujoo, K., et al. Disialoganglioside **GD2** on human neuroblastoma cells. Target antigen for monoclonal antibody-mediated cytolysis and suppression of tumor growth. Cancer Res. 47:1098-1104, 1987.

DETD Cheung, N.-K. V., et al. Ganglioside **GD2** specific monoclonal antibody 3F8. a Phase I study in patients with neuroblastoma and malignant melanoma. J. Clin. Oncol. 5:1430-1440, 1987.

DETD . . . R. F. and Morton, D. L. Regression of cutaneous metastatic melanoma by intralesional injection with human monoclonal antibody to ganglioside **GD2**. Proc. Natl. Acad. Sci. U.S.A. 83:8694-8698, 1986.

DETD Saleh, M. N., et al. Phase I of the murine monoclonal **anti-GD2** antibody 14G2a in metastatic melanoma. Cancer Res. 52: 4342-4347, 1992.

DETD Cheung, N.-K. Cheung, et al. Antibody response to murine **anti-GD2** monoclonal antibodies: Correlation with patient survival. Cancer Res. 54:2228-2233, 1994.

DETD Handgretinger, R., et al. A Phase I study of neuroblastoma with the anti-ganglioside **GD2** antibody 14G2a. Cancer Immunol. Immunother. 35:199-204, 1992.

DETD Cheresh, D. A., et al. Localization of the gangliosides **GD2** and G.sub.p3 in adhesion plaques and on the surface of human melanoma cells. Proc. Natl. Sci. U.S.A., 81:5767-5771, 1984.

DETD Kaufman, H., et al. A recombinant vaccinia virus expressing human carcinoembryonic antigen (**GD2**). Int. J. Cancer, 48:900-907, 1991.

DETD Sen, G., et al. Murine Monoclonal Antibody-idiotype Antibody Breaks Tolerance and Induces Specific Antibody Response to Human Disialoganglioside **GD2** in Cynomolgus Monkeys. Abstract presented at the 9th International Congress of Immunology, San Francisco, Calif., Jul. 23-29, A5250, p885, 1995.

DETD . . . N., Stapleton, J. D., Khazaeli M. B. and LoBuglio, A. F. Generation of a human anti-idiotypic antibody that mimics the **GD2** antigen. J. Immunol. 151:33909-3398, 1993.

CLM What is claimed is:

1. A polynucleotide comprising a sequence encoding a polypeptide that is capable of eliciting an **anti-GD2** immunological response in a mammal, wherein the polypeptide comprises an immunoglobulin variable region containing the three light chain complementarity determining. . .

. . . an amount of the polynucleotide of claim 1 comprising a sequence encoding a polypeptide that is capable of eliciting an **anti-GD2** immunological response in a mammal, the amount being sufficient to elicit an **anti-GD2** immunological response in a human.

15. A method for preparing a polypeptide capable of eliciting an **anti-GD2** immunological response in a mammal, comprising expressing the polynucleotide of claim 1 in a host cell, wherein the polynucleotide is. . .

22. A polynucleotide according to claim 1, wherein the **anti-GD2** immunological response comprises production of **anti-GD2** antibody by the mammal.

23. A polynucleotide according to claim 1, wherein the **anti-GD2** immunological response comprises production of **anti-GD2** reactive T cells by the mammal.

=> d bib abs kwic l15 5

L15 ANSWER 5 OF 10 USPATFULL
AN 1999:12558 USPATFULL
TI Compositions for targeting the vasculature of solid tumors
IN Thorpe, Philip E., Dallas, TX, United States
Burrows, Francis J., San Diego, CA, United States
PA Board of Regents, The University of Texas System, Austin, TX, United States (U.S. corporation)
PI US 5863538 19990126
AI US 1995-457487 19950601 (8)
RLI Division of Ser. No. US 1994-350212, filed on 5 Dec 1994 which is a continuation-in-part of Ser. No. US 1994-205330, filed on 2 Mar 1994, now patented, Pat. No. US 5855866 which is a continuation-in-part of Ser. No. US 1992-846349, filed on 5 Mar 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Feisee, Lila; Assistant Examiner: Bansal, Geetha P.
LREP Arnold, White & Durkee, P.C.
CLMN Number of Claims: 23
ECL Exemplary Claim: 1
DRWN 37 Drawing Figure(s); 25 Drawing Page(s)
LN.CNT 5977
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates generally to methods and compositions for targeting the vasculature of solid tumors using immunological- and growth factor-based reagents. In particular aspects, antibodies carrying diagnostic or therapeutic agents are targeted to the vasculature of solid tumor masses through recognition of tumor vasculature-associated antigens, such as, for example, through endoglin binding, or through the specific induction of endothelial cell surface antigens on vascular endothelial cells in solid tumors.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
SUMM . . . CD14 and FcR for IgE, which will activate the release of IL-1 and TNF.alpha.; and CD16, CD2 or CD3 or **CD28**, which will activate the release of IFN.gamma. and TNF.beta., respectively.
SUMM . . . for IgE, found on Mast cells; FcR for IgG (CD16), found on NK cells; as well as CD2, CD3 or **CD28**, found on the surfaces of T cells. Of these, CD14 targeting will be the most preferred due to the relative. . .
SUMM . . . other cytokines. Thus, for the practice of this aspect of the invention, one will desire to select CD2, CD3 or **CD28** (most preferably **CD28**) as the cytokine activating antigen.
SUMM In particular embodiments, the activating antigen induced by the bispecific antibody will be CD2, CD3, CD14, CD16, FcR for IgE, **CD28** or the T-cell receptor antigen, as may be the case. However, preferably, the bispecific antibody will recognize CD14, and induce the expression of IL-1 by monocyte/macrophage cells in the tumor, or recognize **CD28** and induce the expression of IFN-.gamma. by T-cells in the tumor. Where IL-1 is the cytokine intermediate, the second antibody. . .
DETD . . . bispecific antibodies such as these is predicated in part on the fact that cross-linking antibodies recognizing CD3, CD14, CD16 and **CD28** have previously been shown to elicit cytokine production selectively upon cross-linking with the second antigen (Qian et al., 1991). In. . .
DETD . . . vascular endothelium. Alternatively, the bispecific antibody may be targeted to FcR for IgE, FcR for IgG (CD16), CD2, CD3, or **CD28**, and achieve a similar result, with the cytokine intermediate and cytokine-producing leukocyte being different or the same.
DETD . . . Adhesion Endotoxin)
macrophages
CD14
Molecule-1 Molecule-110
IL-1, TNF-.alpha.
mast cells FcR for IgE
(INCAM-110) TNF-.beta., IL-4

				helper T cells	
					CD2, CD3, **CD28**
		(Immunoglobulin			
			TNF	NK cells	FcR for IgG (CD16)
		Family)			
Intercellular					
	ICAM-1				
		--		IL-1, TNF.alpha.	
				monocytes	CD14
Adhesion		(Immunoglobulin			
			(Bacterial		
				macrophages	
					CD15
Molecule-1		Family)	Endotoxin)		
				mast cells	FcR for IgE
			TNF-.beta., IFN.gamma.		
				T helper cells	
					CD2, CD3, **CD28**
				NK cells	FcR for IgG (CD16)
The Agent for					
	LAM-1 MEL-14 Agent				
			IL-1, TNF.alpha.		
				monocytes	CD14
Leukocytes					
	Agent (Mouse)		(Bacterial		
				macrophages	
					CD14
Adhesion			Endotoxin)		
				mast cells	FcR for IgE
Molecule-1					
Major	MHC	HLA-DR	IFN-.gamma.		
				helper T cells	
					CD2, CD3, **CD28**
Histocompatibility					
	Class	HLA-DP - Human			
Complex	II	HLA-DQ			
Class II		I-A - Mouse		NK cells	FcR for IgG (CD16)
Antigen		I-E			

DETD . . . antibody against a solid tumor antigen that activates T.sub.h 1 cells in the tumor in a CsA-independent fashion, such as **CD28**. Such an antibody will trigger the release of IFN-.gamma. which, in turn, will result in the selective expression of Class. . .

DETD . . . be more suitable for the MHC Class II approach involving, e.g., the cross-linking of T-cells in the tumor through an anti-**CD28** /anti-tumor bispecific antibody, because these tumors are more likely to be infiltrated by T cells, a prerequisite. Examples of immunogenic solid. . .

DETD . . . the tumor. A bispecific (Fab'-Fab') antibody having one arm directed against a tumor antigen and the other arm directed against **CD28** should localize in the tumor and then crosslink **CD28** antigens on T cells in the tumor. Crosslinking of **CD28**, combined with a second signal (provided, for example, by IL-1 which is commonly secreted by tumor cells (Burrows et al.,. . .

DETD . . . involves the preparation of peptic F(ab'.gamma.).sub.2 fragments from the two chosen antibodies (e.g., an antitumor antibody and an anti-CD14 or anti-**CD28** antibody), followed by reduction of each to provide separate Fab'.gamma..sub.SH fragments. The SH groups on one of the two partners. . .

DETD . . . various cytokine activating molecules is also well known in the art. For example, the preparation and use of anti-CD14 and anti-**CD28** monoclonal antibodies having the ability to induce cytokine production by leukocytes has now been described by several laboratories (reviewed in. . .

DETD . . . Int. J. Cancer, 27:775, 1981

glioblastomas			
bladder &	"Ca Antigen"		
	CA1	Ashall et al., Lancet, July 3, 1, 1962	
laryngeal cancers			

350-390 kD
neuroblastoma
GD2 3F8 Cheung et al., Proc. AACR, 27:318, 1986
prostate gp4848 kD GP, 4F.sub.7 /7A.sub.10 Bhattacharya et al., Cancer Res. 44:4528, 1984

Prostate. . .
DETD . . . to the extent that Class II disappears from the vasculature. The mice will then be injected with a bispecific (Fab'--Fab') anti-**CD28**/anti-Ly6A.2 antibody, which should localize to the tumor by virtue of its Ly6.2-binding activity. The bispecific antibody should then bind to T cells which are present in (or which subsequently infiltrate (Blanchard et al., 1988) the tumor. Crosslinking of **CD28** antigens on the T cells by multiple molecules of bispecific antibody attached to the tumor cells should activate the T cells via the CsA-resistant **CD28** pathway (Hess et al., 1991; June et al., 1987; Bjorndahl et al., 1989). Activation of T cells should not occur elsewhere because the crosslinking of **CD28** antigens which is necessary for activation (Thompson et al., 1989; Koulova et al., 1991) should not occur with soluble, non-tumor. . .

DETD An appropriate anti-mouse **CD28** antibody (Gross, et al., 1990) is that obtainable from Dr. James Allison (University of California, Calif.). Ascitic fluid from hybridoma-bearing. . .

DETD . . . by Ghetie, et al. (1988). The ability of the purified anti-Ly6A.2 antibody to bind to MM102 cells and of the anti-**CD28** antibody to bind mouse T cells will be confirmed by FACS analyses as described by Burrows et al., (1991).

DETD F(ab').sub.2 fragments of purified anti-Ly6A.2 and anti-**CD28** antibodies will be prepared by pepsin digestion, as described by Glennie et al. (1987). Purified antibodies (5-10 mg) will be. . .

DETD d) Preparation of anti-Ly6A.2/anti-**CD28** Bispecific Antibodies

DETD For the production of anti-Ly6A.2-anti-**CD28** bispecific antibodies, Fab' fragments of each antibody will be initially prepared as above and will be left unalkylated. Heterodimer molecules. . .

DETD e) Confirmation of Cell Binding-Capacity of Anti-Ly6A.2/Anti-**CD28** Bispecific-Antibody

DETD . . . that the bispecific antibody is intact and is capable of binding tumor cells. The study will be repeated using a **CD28** positive mouse T cell lymphoma line (e.g., EL4) and with fluoresceinated goat anti-mouse immunoglobulin as the detecting antibody to confirm that the bispecific antibody has **CD28**-binding capacity.

DETD f) Activation of T Cells by Anti-Ly6A.2/Anti-**CD28** Bispecific Antibody Plus MM102 Tumor Cells

DETD . . . cells will be cultured (0.5 to 1.times.10.sup.5 cells/0.2 ml) in medium in the wells of 96-well plates. Various concentrations of anti-**CD28** IgG, anti-**CD28** Fab' or anti-Ly6A.2/anti-**CD28** bispecific antibody will be added together with various concentrations of one of the following costimulants: PMA, IL1 or anti-CD3 IgG.. . . .sup.3 H-thymidine (1 .mu.Ci/culture) will be added and the plates harvested 24 hours later. These studies should confirm that bivalent anti-**CD28**, but not monovalent Fab' anti-**CD28** or the bispecific antibody, stimulate T cells and that the stimulation is not CsA inhibitable.

DETD g) Confirmation that Injection of Anti-Ly6A.2/Anti-**CD28** Bispecific Antibody into CsA-Treated MM102 Tumor-Bearing Mice Results in Induction of Class II Selectively on Tumor Vasculature

DETD . . . in diameter, and when Class II will have disappeared from the vasculature, mice will be injected with 50-100 .mu.g of anti-Ly6A.2/anti-**CD28** bispecific antibody. Other mice will receive various control treatments, including unconjugated anti-Ly6A.2 or anti-**CD28** (Fab' and IgG) or diluent alone. Two or three days later, the mice will be sacrificed and the tumors and. . .

DETD . . . IFN-.gamma. secretion to ensue. If so, the presence of T cells will be verified by staining frozen tumor sections with anti-**CD28** and anti-CD3 antibodies. If T cells are present, again as would be anticipated from prior studies (Koulova, et al., 1991;. . . a 2nd signal might be missing. This will be checked by coadministering an anti-Ly6A.2/anti-CD3 bispecific antibody, which together with the

anti-Ly6A.2/anti-**CD28** bispecific, should provide the signalling needed for T cell activation.

DETD . . . mg/kg/d). When the tumors have grown to 1.0-1.3 cm diameter, the mice will receive an intravenous injection of 50-100 .mu.g anti-Ly6A.2/anti-**CD28** bispecific antibody (perhaps together with anti-Ly6A.2/anti-CD3 bispecific antibody if indicated by the studies in Section (h) above). Two or three. . . comparing the anti-tumor effects with those in mice which receive various control treatments, including unconjugated anti-Ly6A.2 Fab' and IgG, unconjugated anti-**CD28** Fab' and IgG, and anti-Class II immunotoxin alone.

CLM What is claimed is:

. . . 1, wherein the first antibody binds to the leukocyte cell surface activating antigen CD2, CD3, CD14, CD16, FcR for IgE, **CD28** or the T-cell receptor antigen.

5. The combination of claim 2, wherein the first antibody binds to **CD28**, and induces the expression of the cytokine IFN-.gamma. by T-cells.

8. The combination of claim 2, wherein the first antibody binds to CD14 or **CD28** and to a tumor antigen.

=> d bib abs kwic l15 6

L15 ANSWER 6 OF 10 USPATFULL
AN 1999:1209 USPATFULL
TI Methods for treating the vasculature of solid tumors
IN Thorpe, Philip E., Dallas, TX, United States
Burrows, Francis J., Dallas, TX, United States
PA Board of Regenis, The University of Texas System, Austin, TX, United States (U.S. corporation)
PI US 5855866 19990105
AI US 1994-205330 19940302 (8)
RLI Continuation-in-part of Ser. No. US 1992-846349, filed on 5 Mar 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Kight, John; Assistant Examiner: Hartley, Michael G.
LREP Arnold, White & Durkee
CLMN Number of Claims: 26
ECL Exemplary Claim: 1
DRWN 19 Drawing Figure(s); 25 Drawing Page(s)
LN.CNT 5207
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates generally to methods and compositions for targeting the vasculature of solid tumors using immunologically-based reagents. In particular aspects, antibodies carrying diagnostic or therapeutic agents are targeted to the vasculature of solid tumor masses through recognition of tumor vasculature-associated antigens, such as, for example, through endoglin binding, or through the specific induction of endothelial cell surface antigens on vascular endothelial cells in solid tumors.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
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SUMM . . . for IgE, found on Mast cells; FcR for IgG (CD16), found on NK cells; as well as CD2, CD3 or **CD28**, found on the surfaces of T cells. Of these, CD14 targeting will be the most preferred due to the relative. . .
SUMM . . . other cytokines. Thus, for the practice of this aspect of the invention, one will desire to select CD2, CD3 or **CD28** (most preferably **CD28**) as the cytokine activating antigen.
SUMM In particular embodiments, the activating antigen induced by the bispecific antibody will be CD2, CD3, CD14, CD16, FcR for IgE, **CD28** or the T-cell receptor antigen, as may be the case. However, preferably, the bispecific antibody will recognize CD14, and induce the expression of IL-1 by monocyte/macrophage cells in the tumor, or recognize **CD28** and induce the expression of IFN-.gamma. by T-cells in the tumor. Where IL-1 is the cytokine intermediate, the second antibody. . .
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DETD . . . vascular endothelium. Alternatively, the bispecific antibody may be targeted to FcR for IgE, FcR for IgG (CD16), CD2, CD3, or **CD28**, and achieve a similar result, with the cytokine intermediate and cytokine-producing leukocyte being different or the same.
DETD . . . macrophages
CD14
Molecule-1 Molecule-110
IL-1, TNF-
mast cells FcR for IgE
(INCAM-110)
.alpha.
(Immunoglobulin
TNF-.beta., IL-

				helper T cells	
					CD2, CD3, **CD28**
	Family)	4			
		TNF	NK cells	FcR for IgG (CD16)	
Intercellular					
ICAM-1	--	IL-1, TNF.alpha.			
				monocytes	CD14
Adhesion	(Immunoglobulin				
		(Bacterial			
				macrophages	
					CD15
Molecule-1	Family)	Endotoxin)			
				mast cells	FcR for IgE
		TNF-.beta.,			
				T helper cells	
					CD2, CD3, **CD28**
		IFN.gamma.			
				NK cells	FcR for IgG (CD16)
The Agent for					
LAM-1	MEL-14 Agent				
		Il-1, TNF.alpha.			
				monocytes	CD14
Leukocyte Agent	(Mouse)	(Bacterial			
				macrophages	
					CD14
Adhesion		Endotoxin)			
				mast cells	FcR for IgE
Molecule-1					
Major MHC	HLA-DR	IFN-.gamma.			
				helper T cells	
					CD2, CD3, **CD28**
Histocompata-					
Class	HLA-DP - Human				
bility Complex					
II	HLA-DQ				
Class II	I-A - Mouse		NK cells	FcR for IgG (CD16)	
Antigen	I-E				

DETD . . . antibody against a solid tumor antigen that activates T.sub.h 1 cells in the tumor in a CsA-independent fashion, such as **CD28**. Such an antibody will trigger the release of IFN-1 which, in turn, will result in the selective expression of Class. . .

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glioblastomas		
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GD2 3F8 Cheung et al., Proc. AACR, 27:318, 1986
Prostate gp48 48 kD GP
4F.sub.7 /7A.sub.10
Bhattacharya et al., Cancer Res. 44:4528,

. . .

DETD . . . to the extent that Class II disappears from the vasculature. The mice will then be injected with a bispecific (Fab'-Fab') anti-**CD28**/anti-Ly6A.2 antibody, which should localize to the tumor by virtue of its Ly6.2-binding activity. The bispecific antibody should then bind to T cells which are present in (or which subsequently infiltrate (Blanchard et al., 1988) the tumor. Crosslinking of **CD28** antigens on the T cells by multiple molecules of bispecific antibody attached to the tumor cells should activate the T cells via the CsA-resistant **CD28** pathway (Hess et al., 1991; June et al., 1987; Bjorndahl et al., 1989). Activation of T cells should not occur elsewhere because the crosslinking of **CD28** antigens which is necessary for activation (Thompson et al., 1989; Koulova et al., 1991) should not occur with soluble, non-tumor. . .

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DETD F(ab').sub.2 fragments of purified anti-Ly6A.2 and anti-**CD28** antibodies will be prepared by pepsin digestion, as described by Glennie et al. (1987). Purified antibodies (5-10 mg) will be. . .

DETD d) Preparation of anti-Ly6A.2/anti-**CD28** bispecific antibodies

DETD For the production of anti-Ly6A.2-anti-**CD28** bispecific antibodies, Fab' fragments of each antibody will be initially prepared as above and will be left unalkylated. Heterodimer molecules. . .

DETD e) Confirmation of cell binding-capacity of anti-Ly6A.2/anti-**CD28** bispecific-antibody

DETD . . . that the bispecific antibody is intact and is capable of binding tumor cells. The study will be repeated using a **CD28** positive mouse T cell lymphoma line (e.g., EL4) and with fluoresceinated goat anti-mouse immunoglobulin as the detecting antibody to confirm that the bispecific antibody has **CD28**-binding capacity.

DETD f) Activation of T cells by anti-Ly6A.2/anti-**CD28** bispecific antibody plus MM102 tumor cells

DETD . . . cells will be cultured (0.5 to 1.times.10.sup.5 cells/0.2 ml) in medium in the wells of 96-well plates. Various concentrations of anti-**CD28** IgG, anti-**CD28** Fab' or anti-Ly6A.2/anti-**CD28** bispecific antibody will be added together with various concentrations of one of the following costimulants: PMA, IL1 or anti-CD3 IgG.. . . .sup.3 H-thymidine (1 .mu.Ci/culture) will be added and the plates harvested 24 hours later. These studies should confirm that bivalent anti-**CD28**, but not monovalent Fab' anti-**CD28** or the bispecific antibody, stimulate T cells and that the stimulation is not CsA inhibitable.

DETD g) Confirmation that injection of anti-Ly6A.2/anti-**CD28** bispecific antibody into CsA-treated MM102 tumor-bearing mice results in induction of Class II selectively on tumor vasculature

DETD . . . in diameter, and when Class II will have disappeared from the vasculature, mice will be injected with 50-100 .mu.g of anti-Ly6A.2/anti-**CD28** bispecific antibody. Other mice will receive various control treatments, including unconjugated anti-Ly6A.2 or anti-**CD28** (Fab' and IgG) or diluent alone. Two or three days later, the mice will be sacrificed and the tumors and. . .

DETD . . . IFN-.gamma. secretion to ensue. If so, the presence of T cells will be verified by staining frozen tumor sections with anti-**CD28** and anti-CD3 antibodies. If T cells are present, again as would be anticipated from prior studies (Koulova, et al., 1991;. . . a 2nd signal might be missing. This will be checked by coadministering an anti-Ly6A.2/anti-CD3 bispecific antibody, which together with the anti-Ly6A.2/anti-**CD28** bispecific, should provide the signalling needed for T cell activation.

DETD . . . mg/kg/d). When the tumors have grown to 1.0-1.3 cm diameter, the mice will receive an intravenous injection of 50-100 .mu.g

anti-Ly6A.2/anti-**CD28** bispecific antibody (perhaps together with anti-Ly6A.2/anti-CD3 bispecific antibody if indicated by the studies in Section (h) above). Two or three. . . comparing the anti-tumor effects with those in mice which receive various control treatments, including unconjugated anti-Ly6A.2 Fab' and IgG, unconjugated anti-**CD28** Fab' and IgG, and anti-Class II immunotoxin alone.

=> d bib abs kwic l15 7

L15 ANSWER 7 OF 10 USPATFULL
AN 1998:147225 USPATFULL
TI Methods for enriching specific cell-types by density gradient centrifugation
IN Van Vlasselaer, Peter, Sunnyvale, CA, United States
PA Activated Cell Therapy, Inc., Mountain View, CA, United States (U.S. corporation)
PI US 5840502 19981124
AI US 1994-299467 19940831 (8)
DT Utility
EXNAM Primary Examiner: Green, Lora M.; Assistant Examiner: Wolski, Susan C.
LREP Stratford, Carol A.; Glaister, Debra J.; Dehlinger, Peter J.
CLMN Number of Claims: 13
ECL Exemplary Claim: 7
DRWN 53 Drawing Figure(s); 16 Drawing Page(s)
LN.CNT 2018
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to methods of enriching for desired cell population from cell sources, such as body fluids, dispersed tissue specimens and cultured cells. In particular, the present invention relates to the use of a cell-trap centrifugation tube containing a specific density gradient solution adjusted to the specific density of a desired cell population to enrich for the desired cell from a cell source. The tube allows the desired cell population to be collected by decantation after centrifugation to minimize cell loss and maximize efficiency. In addition, the method can be further simplified by density-adjusted cell sorting which uses cell type-specific binding agents such as antibodies and lectins linked to carrier particles to impart a different density to the undesired populations in a more convenient manner. The rapid cell enrichment method described herein has a wide range of diagnostic and therapeutic applications.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
DETD . . . et al., 1977) associated with prostate cancer; gp75/brown (Brichard et al., 1993, J. Exp. Med. 178:489 associated with melanoma; gangliosides (GM2,**GD2**) (Lloyd, 1991, Seminars in Cancer Biology 2:421) associated with melanoma; melanotransferrin (Real et al., 1984, J. Exp. Med. 160:1219); p53,. . .
DETD . . . anti-CD24 specific for B-cells and granulocytes; anti-CD25 and anti-CD26 specific for activated T- and B-cells and activated macrophages; anti-CD27 and anti-**CD28** specific for major T-cell subset; anti-CD30 specific for activated T- and B-cells and Sternberg Reed cells; anti-CD31 specific for platelets,. . .

=> d bib abs kwic l15 8

L15 ANSWER 8 OF 10 USPATFULL
AN 1998:112052 USPATFULL
TI Immunogenic peptides of prostate specific antigen
IN Kokolus, William J., 7900 Cambridge St. #14-2L, Houston, TX, United States 77054
Fritsche, Herbert A., 4506 Frontier, Houston, TX, United States 77041
Johnston, Dennis A., 2010 Ramada Dr., Houston, TX, United States 77062
PI US 5807978 19980915
AI US 1995-472228 19950607 (8)
DT Utility
EXNAM Primary Examiner: Scheiner, Toni R.; Assistant Examiner: Johnson, Nancy A.
LREP Fuierer, Marianne; Ellis, Howard M.
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 1 Drawing Figure(s); 1 Drawing Page(s)
LN.CNT 1657
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Peptides derived from prostate specific antigen (PSA) that correspond to the immunodominant epitopes found in the native antigen are disclosed. These peptides were identified using a method that predicts continuous, immunodominant epitopes. Anti-PSA antibodies, methods for their production and their use in diagnostic assays also are disclosed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
DETD Cluster Differentiation Antigens and MHC Antigens: CD2, CD3, CD4, CD5, CD8, CD11a, CD11b, CD11c, CD16, CD18, CD21, **CD28**, CD32, CD34, CD35, CD40, CD44, CD45, CD54, CD56, K2, K1, P.beta., O.alpha., M.alpha., M.beta.2, M.beta.1, LMP1, TAP2, LMP7, TAP1, O.beta.,. . .
DETD Tumor Markers and Tumor Suppressors: .beta.-HCG, 4-N-acetylgalactosaminyltransferase, GM2, **GD2**, GD3, MAGE-1, MAGE-2, MAGE-3, MUC-1, MUC-2, MUC-3, MUC-4, MUC-18, ICAM-1, C-CAM, V-CAM, ELAM, NM23, EGFR, E-cadherin, N-CAM, CEA, DCC, PSA,. . .

=> d bib abs kwic l15 9

L15 ANSWER 9 OF 10 USPATFULL
AN 1998:78687 USPATFULL
TI Methods for targeting the vasculature of solid tumors
IN Thorpe, Philip E., Dallas, TX, United States
Burrows, Francis J., San Diego, CA, United States
PA Board of Regents, The University of Texas System, Austin, TX, United States (U.S. corporation)
PI US 5776427 19980707
AI US 1995-456495 19950601 (8)
RLI Division of Ser. No. US 1994-350212, filed on 5 Dec 1994 which is a continuation-in-part of Ser. No. US 1994-205330, filed on 2 Mar 1994 which is a continuation-in-part of Ser. No. US 1992-846349, filed on 5 Mar 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Kight, John; Assistant Examiner: Hartley, Michael G.
LREP Arnold, White & Durkee
CLMN Number of Claims: 23
ECL Exemplary Claim: 1
DRWN 27 Drawing Figure(s); 25 Drawing Page(s)
LN.CNT 5943
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates generally to methods and compositions for targeting the vasculature of solid tumors using immunological- and growth factor-based reagents. In particular aspects, antibodies carrying diagnostic or therapeutic agents are targeted to the vasculature of solid tumor masses through recognition of tumor vasculature-associated antigens, such as, for example, through endoglin binding, or through the specific induction of endothelial cell surface antigens on vascular endothelial cells in solid tumors.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
SUMM . . . CD14 and FcR for IgE, which will activate the release of IL-1 and TNF.alpha.; and CD16, CD2 or CD3 or **CD28**, which will activate the release of IFN.gamma. and TNF.beta., respectively.
SUMM . . . for IgE, found on Mast cells; FcR for IgG (CD16), found on NK cells; as well as CD2, CD3 or **CD28**, found on the surfaces of T cells. Of these, CD14 targeting will be the most preferred due to the relative. . .
SUMM . . . other cytokines. Thus, for the practice of this aspect of the invention, one will desire to select CD2, CD3 or **CD28** (most preferably **CD28**) as the cytokine activating antigen.
SUMM In particular embodiments, the activating antigen induced by the bispecific antibody will be CD2, CD3, CD14, CD16, FcR for IgE, **CD28** or the T-cell receptor antigen, as may be the case. However, preferably, the bispecific antibody will recognize CD14, and induce the expression of IL-1 by monocyte/macrophage cells in the tumor, or recognize **CD28** and induce the expression of IFN-.gamma. by T-cells in the tumor. Where IL-1 is the cytokine intermediate, the second antibody. . .
DETD . . . bispecific antibodies such as these is predicated in part on the fact that cross-linking antibodies recognizing CD3, CD14, CD16 and **CD28** have previously been shown to elicit cytokine production selectively upon cross-linking with the second antigen (Qian et al., 1991). In. . .
DETD . . . vascular endothelium. Alternatively, the bispecific antibody may be targeted to FcR for IgE, FcR for IgG (CD16), CD2, CD3, or **CD28**, and achieve a similar result, with the cytokine intermediate and cytokine-producing leukocyte being different or the same.
DETD . . . macrophages
CD14
Molecule-1 Molecule-110
IL-1, TNF-
mast cells
FcR for IgE
(INCAM-119)

.alpha.
(Immunoglobulin
TNF-.beta., IL-4
helper T cells
CD2, CD3, **CD28**
Family) TNF NK cells FcR for IgG (CD16)
Intercellular
ICAM-1
-- IL-1, TNF.alpha.
monocytes
CD14
Adhesion (Immunoglobulin
(Bacterial
macrophages
CD15
Molecule-1 Family) Endotoxin)
mast cells
FcR for IgE
TNF-.beta.,
T helper cells
CD2, CD3, **CD28**
IFN.gamma.
NK cells FcR for IgG (CD16)
The Agent for
LAM-1 MEL-14 Agent
Il-1, TNF.alpha.
monocytes
CD14
Leukocyte Agent (Mouse) (Bacterial
macrophages
CD14
Adhesion Endotoxin)
mast cells
FcR for IgE
Molecule-1
Major MHC HLA-DR -
Human
IFN-.gamma.
helper T cells
CD2, CD3, **CD28**
Histocompatibility
Class HLA-DP
Complex II HLA-DQ
Class II I-A -
Mouse NK cells FcR for IgG (CD16)
Antigen I-E

DETD . . . antibody against a solid tumor antigen that activates T.sub.h 1 cells in the tumor in a CsA-independent fashion, such as **CD28**. Such an antibody will trigger the release of IFN-.gamma. which, in turn, will result in the selective expression of Class. . .

DETD . . . be more suitable for the MHC Class II approach involving, e.g., the cross-linking of T-cells in the tumor through an anti-**CD28** /anti-tumor bispecific antibody, because these tumors are more likely to be infiltrated by T cells, a prerequisite. Examples of immunogenic solid. . .

DETD . . . the tumor. A bispecific (Fab'-Fab') antibody having one arm directed against a tumor antigen and the other arm directed against **CD28** should localize in the tumor and then crosslink **CD28** antigens on T cells in the tumor. Crosslinking of **CD28**, combined with a second signal (provided, for example, by IL-1 which is commonly secreted by tumor cells (Burrows et al.,. . .

DETD . . . involves the preparation of peptic F(ab'.gamma.).sub.2 fragments from the two chosen antibodies (e.g., an antitumor antibody and an anti-CD14 or anti-**CD28** antibody), followed by reduction of each to provide separate Fab'.gamma.SH fragments. The SH groups on one of the two partners. . .

DETD . . . various cytokine activating molecules is also well known in the art. For example, the preparation and use of anti-CD14 and anti-**CD28** monoclonal antibodies having the ability to induce cytokine

production by leukocytes has now been described by several laboratories (reviewed in. . .

DETD . . . Int. J. Cancer, 27:775, 1981

glioblastomas
bladder & "Ca Antigen"
CA1 Ashall et al., Lancet, July 3, 1, 1982
laryngeal cancers
350-390 kD
neuroblastoma
GD2 3F8 Cheung et al., Proc. AACR, 27:318, 1986
Prostate gp48 48 kD GP
4F.sub.7 /7A.sub.10
Bhattacharya et al., Cancer Res. 44:4528,. . .

DETD . . . to the extent that Class II disappears from the vasculature. The mice will then be injected with a bispecific (Fab'-Fab') anti-**CD28**/anti-Ly6A.2 antibody, which should localize to the tumor by virtue of its Ly6.2-binding activity. The bispecific antibody should then bind to T cells which are present in (or which subsequently infiltrate (Blanchard et al., 1988) the tumor. Crosslinking of **CD28** antigens on the T cells by multiple molecules of bispecific antibody attached to the tumor cells should activate the T cells via the CsA-resistant **CD28** pathway (Hess et al., 1991; June et al., 1987; Bjorndahl et al., 1989). Activation of T cells should not occur elsewhere because the crosslinking of **CD28** antigens which is necessary for activation (Thompson et al., 1989; Koulova et al., 1991) should not occur with soluble, non-tumor. . .

DETD An appropriate anti-mouse **CD28** antibody (Gross, et al., 1990) is that obtainable from Dr. James Allison (University of California, CA). Ascitic fluid from hybridoma-bearing. . .

DETD . . . by Ghetie, et al. (1988). The ability of the purified anti-Ly6A.2 antibody to bind to MM102 cells and of the anti-**CD28** antibody to bind mouse T cells will be confirmed by FACS analyses as described by Burrows et al., (1991).

DETD F(ab').sub.2 fragments of purified anti-Ly6A.2 and anti-**CD28** antibodies will be prepared by pepsin digestion, as described by Glennie et'al. (1987). Purified antibodies (5-10 mg) will be dialyzed. . .

DETD d) Preparation of anti-Ly6A.2/anti-**CD28** bispecific antibodies

DETD For the production of anti-Ly6A.2-anti-**CD28** bispecific antibodies, Fab' fragments of each antibody will be initially prepared as above and will be left unalkylated. Heterodimer molecules. . .

DETD e) Confirmation of cell binding-capacity of anti-Ly6A.2/anti-**CD28** bispecific-antibody

DETD . . . that the bispecific antibody is intact and is capable of binding tumor cells. The study will be repeated using a **CD28** positive mouse T cell lymphoma line (e.g., EL4) and with fluoresceinated goat anti-mouse immunoglobulin as the detecting antibody to confirm that the bispecific antibody has **CD28**-binding capacity.

DETD f) Activation of T cells by anti-Ly6A.2/anti-**CD28** bispecific antibody plus MM102 tumor cells

DETD . . . cells will be cultured (0.5 to 1.times.10.sup.5 cells/0.2 ml) in medium in the wells of 96-well plates. Various concentrations of anti-**CD28** IgG, anti-**CD28** Fab' or anti-Ly6A.2/anti-**CD28** bispecific antibody will be added together with various concentrations of one of the following costimulants: PMA, IL1 or anti-CD3 IgG.. . . .sup.3 H-thymidine (1 .mu.Ci/culture) will be added and the plates harvested 24 hours later. These studies should confirm that bivalent anti-**CD28**, but not monovalent Fab' anti-**CD28** or the bispecific antibody, stimulate T cells and that the stimulation is not CsA inhibitable.

DETD g) Confirmation that injection of anti-Ly6A.2/anti-**CD28** bispecific antibody into CsA-treated MM102 tumor-bearing mice results in induction of Class II selectively on tumor vasculature

DETD . . . in diameter, and when Class II will have disappeared from the vasculature, mice will be injected with 50-100 .mu.g of anti-Ly6A.2/anti-**CD28** bispecific antibody. Other mice will receive various control treatments, including unconjugated anti-Ly6A.2 or anti-**CD28** (Fab' and IgG) or diluent alone. Two or three

days later, the mice will be sacrificed and the tumors and. . .

DETD . . . IFN-.gamma. secretion to ensue. If so, the presence of T cells will be verified by staining frozen tumor sections with anti-**CD28** and anti-CD3 antibodies. If T cells are present, again as would be anticipated from prior studies (Koulova, et al., 1991;. . . a 2nd signal might be missing. This will be checked by coadministering an anti-Ly6A.2/anti-CD3 bispecific antibody, which together with the anti-Ly6A.2/anti-**CD28** bispecific, should provide the signalling needed for T cell activation.

DETD . . . mg/kg/d). When the tumors have grown to 1.0-1.3 cm diameter, the mice will receive an intravenous injection of 50-100 .mu.g anti-Ly6A.2/anti-**CD28** bispecific antibody (perhaps together with anti-Ly6A.2/anti-CD3 bispecific antibody if indicated by the studies in Section (h) above). Two or three. . . comparing the anti-tumor effects with those in mice which receive various control treatments, including unconjugated anti-Ly6A.2 Fab' and IgG, unconjugated anti-**CD28** Fab' and IgG, and anti-Class II immunotoxin alone.

CLM What is claimed is:

. . . wherein the first bispecific antibody binds to the leukocyte cell surface activating antigen CD2, CD3, CD14, CD16, FcR for IgE, **CD28** or the T-cell receptor antigen.

7. The method of claim 4, wherein the first bispecific antibody binds to CD14 or **CD28** and to a tumor antigen.

8. The method of claim 7, wherein the first bispecific antibody binds to CD14 or **CD28** and to a human tumor-associated antigen selected from the group consisting of p185.sup.HER2, milk mucin core protein, TAG-72, Lewis a,. . .

11. The method of claim 4, wherein the first bispecific antibody binds to **CD28** and induces the expression of the cytokine IFN-.gamma. by T-cells in the tumor.

=> d bib abs kwic l15 10

L15 ANSWER 10 OF 10 USPATFULL
AN 97:75816 USPATFULL
TI Antibodies that bind to endoglin
IN Thorpe, Philip E., Dallas, TX, United States
Burrows, Francis J., San Diego, CA, United States
PA Board of Regents, The University of Texas System, Austin, TX, United States (U.S. corporation)
PI US 5660827 19970826
AI US 1995-457229 19950601 (8)
RLI Division of Ser. No. US 1994-350212, filed on 5 Dec 1994 which is a continuation-in-part of Ser. No. US 1994-205330, filed on 2 Mar 1994 which is a continuation-in-part of Ser. No. US 1994-295868, filed on 6 Sep 1994 which is a continuation-in-part of Ser. No. US 1992-846349, filed on 5 Mar 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Feisee, Lila; Assistant Examiner: Ebert, Ray F.
LREP Arnold, White & Durkee
CLMN Number of Claims: 30
ECL Exemplary Claim: 1,16
DRWN 37 Drawing Figure(s); 25 Drawing Page(s)
LN.CNT 5787
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are antibodies that specifically bind to endoglin. Conjugates of the antibodies linked to diagnostic or therapeutic agents are also provided. Methods of using the antibodies and conjugates are also disclosed, including methods of targeting the vasculature of solid tumors through recognition of the tumor vasculature-associated antigen, endoglin.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
SUMM . . . CD14 and FcR for IgE, which will activate the release of IL-1 and TNF.alpha.; and CD16, CD2 or CD3 or **CD28**, which will activate the release of IFN.gamma. and TNF.beta., respectively.
SUMM . . . for IgE, found on Mast cells; FcR for IgG (CD16), found on NK cells; as well as CD2, CD3 or **CD28**, found on the surfaces of T cells. Of these, CD14 targeting will be the most preferred due to the relative. . .
SUMM . . . other cytokines. Thus, for the practice of this aspect of the invention, one will desire to select CD2, CD3 or **CD28** (most preferably **CD28**) as the cytokine activating antigen.
SUMM In particular embodiments, the activating antigen induced by the bispecific antibody will be CD2, CD3, CD14, CD16, FcR for IgE, **CD28** or the T-cell receptor antigen, as may be the case. However, preferably, the bispecific antibody will recognize CD14, and induce the expression of IL-1 by monocyte/macrophage cells in the tumor, or recognize **CD28** and induce the expression of IFN-.gamma. by T-cells in the tumor. Where IL-1 is the cytokine intermediate, the second antibody. . .
DETD . . . bispecific antibodies such as these is predicated in part on the fact that cross-linking antibodies recognizing CD3, CD14, CD16 and **CD28** have previously been shown to elicit cytokine production selectively upon cross-linking with the second antigen (Qian et al., 1991). In. . .
DETD . . . vascular endothelium. Alternatively, the bispecific antibody may be targeted to FcR for IgE, FcR for IgG (CD16), CD2, CD3, or **CD28**, and achieve a similar result, with the cytokine intermediate and cytokine-producing leukocyte being different or the same.
DETD . . . macrophages
CD14
Molecule-1 Molecule-110
IL-1, TNF-
mast cells FcR for IgE
(INCAM-110)
.alpha.
(Immunoglobulin

			TNF-.beta., IL-		
				helper T cells	
					CD2, CD3, **CD28**
		Family)	4		
			TNF	NK cells	FcR for IgG (CD16)
Intercellular					
	ICAM-1	--	IL-1, TNF.alpha.		
				monocytes	CD14
Adhesion		(Immunoglobulin			
			(Bacterial		
				macrophages	
					CD15
Molecule-1		Family)	Endotoxin)		
				mast cells	FcR for IgE
			TNF-.beta.,		
				T helper cells	
					CD2, CD3, **CD28**
			IFN.gamma.		
				NK cells	FcR for IgG (CD16)
The Agent for					
	LAM-1	MEL-14 Agent			
			Il-1, TNF.alpha.		
				monocytes	CD14
Leukocyte Agent		(Mouse)	(Bacterial		
				macrophages	
					CD14
Adhesion			Endotoxin)		
				mast cells	FcR for IgE
Molecule-1					
Major	MHC	HLA-DR	IFN-.gamma.		
				helper T cells	
					CD2, CD3, **CD28**
Histocompatibi					
	Class	HLA-DP - Human			
lity Complex					
	II	HLA-DQ			
Class II		I-A - Mouse		NK cells	FcR for IgG (CD16)
Antigen		I-E			

DETD . . . antibody against a solid tumor antigen that activates T.sub.h 1 cells in the tumor in a CsA-independent fashion, such as **CD28**. Such an antibody will trigger the release of IFN-.gamma. which, in turn, will result in the selective expression of Class. . .

DETD . . . be more suitable for the MHC Class II approach involving, e.g., the cross-linking of T-cells in the tumor through an anti-**CD28** /anti-tumor bispecific antibody, because these tumors are more likely to be infiltrated by T cells, a prerequisite. Examples of immunogenic solid. . .

DETD . . . the tumor. A bispecific (Fab'--Fab') antibody having one arm directed against a tumor antigen and the other arm directed against **CD28** should localize in the tumor and then crosslink **CD28** antigens on T cells in the tumor. Crosslinking of **CD28**, combined with a second signal (provided, for example, by IL-1 which is commonly secreted by tumor cells (Burrows et al.,. . .

DETD . . . involves the preparation of peptic F(ab'.gamma.).sub.2 fragments from the two chosen antibodies (e.g., an antitumor antibody and an anti-CD14 or anti-**CD28** antibody), followed by reduction of each to provide separate Fab'.sub..gamma.SH fragments. The SH groups on one of the two partners. . .

DETD . . . various cytokine activating molecules is also well known in the art. For example, the preparation and use of anti-CD14 and anti-**CD28** monoclonal antibodies having the ability to induce cytokine production by leukocytes has now been described by several laboratories (reviewed in. . .

DETD . . . Int. J. Cancer, 27:775, 1981

glioblastomas			
bladder &	"Ca Aotigen"		
	CA1	Ashall et al., Lancet, July 3, 1, 1982	
laryngeal cancers			

350-390 kD
neuroblastoma
GD2 3F8 Cheung et al., Proc. AACR, 27:318, 1986
Prostate gp48 48 kD GP
4F.sub.7 /7A.sub.10
Bhattacharya et al., Cancer Res. 44:4528,

. . .

DETD . . . to the extent that Class II disappears from the vasculature. The mice will then be injected with a bispecific (Fab'--Fab') anti-**CD28**/anti-Ly6A.2 antibody, which should localize to the tumor by virtue of its Ly6.2-binding activity. The bispecific antibody should then bind to T cells which are present in (or which subsequently infiltrate (Blanchard et al., 1988) the tumor. Crosslinking of **CD28** antigens on the T cells by multiple molecules of bispecific antibody attached to the tumor cells should activate the T cells via the CsA-resistant **CD28** pathway (Hess et al., 1991; June et al., 1987; Bjorndahl et al., 1989). Activation of T cells should not occur elsewhere because the crosslinking of **CD28** antigens which is necessary for activation (Thompson et al., 1989; Koulova et al., 1991) should not occur with soluble, non-tumor. . .

DETD An appropriate anti-mouse **CD28** antibody (Gross, et al., 1990) is that obtainable from Dr. James Allison (University of California, California). Ascitic fluid from hybridoma-bearing. . .

DETD . . . by Ghetie, et al. (1988). The ability of the purified anti-Ly6A.2 antibody to bind to MM102 cells and of the anti-**CD28** antibody to bind mouse T cells will be confirmed by FACS analyses as described by Burrows et al., (1991).

DETD F(ab').sub.2 fragments of purified anti-Ly6A.2 and anti-**CD28** antibodies will be prepared by pepsin digestion, as described by Glennie et al. (1987). Purified antibodies (5-10 mg) will be. . .

DETD d) Preparation of anti-Ly6A.2/anti-**CD28** bispecific antibodies

DETD For the production of anti-Ly6A.2-anti-**CD28** bispecific antibodies, Fab' fragments of each antibody will be initially prepared as above and will be left unalkylated. Heterodimer molecules. . .

DETD e) Confirmation of cell binding-capacity of anti-Ly6A.2/anti-**CD28** bispecific-antibody

DETD . . . that the bispecific antibody is intact and is capable of binding tumor cells. The study will be repeated using a **CD28** positive mouse T cell lymphoma line (e.g., EL4) and with fluoresceinated goat anti-mouse immunoglobulin as the detecting antibody to confirm that the bispecific antibody has **CD28**-binding capacity.

DETD f) Activation of T cells by anti-Ly6A.2/anti-**CD28** bispecific antibody plus MM102 tumor cells

DETD . . . cells will be cultured (0.5 to 1.times.10.sup.5 cells/0.2 ml) in medium in the wells of 96-well plates. Various concentrations of anti-**CD28** IgG, anti-**CD28** Fab' or anti-Ly6A.2/anti-**CD28** bispecific antibody will be added together with various concentrations of one of the following costimulants: PMA, IL1 or anti-CD3 IgG.. . . .sup.3 H-thymidine (1 .mu.Ci/culture) will be added and the plates harvested 24 hours later. These studies should confirm that bivalent anti-**CD28**, but not monovalent Fab' anti-**CD28** or the bispecific antibody, stimulate T cells and that the stimulation is not CsA inhibitable.

DETD g) Confirmation that injection of anti-Ly6A.2/anti-**CD28** bispecific antibody into CsA-treated MM102 tumor-bearing mice results in induction of Class II selectively on tumor vasculature

DETD . . . in diameter, and when Class II will have disappeared from the vasculature, mice will be injected with 50-100 .mu.g of anti-Ly6A.2/anti-**CD28** bispecific antibody. Other mice will receive various control treatments, including unconjugated anti-Ly6A.2 or anti-**CD28** (Fab' and IgG) or diluent alone. Two or three days later, the mice will be sacrificed and the tumors and. . .

DETD . . . IFN-.gamma. secretion to ensue. If so, the presence of T cells will be verified by staining frozen tumor sections with anti-**CD28** and anti-CD3 antibodies. If T cells are present, again as would be anticipated from prior studies (Koulova, et al., 1991;. . . a 2nd signal might be missing. This will be checked by coadministering an anti-Ly6A.2/anti-CD3 bispecific antibody, which together with the

anti-Ly6A.2/anti-**CD28** bispecific, should provide the signalling needed for T cell activation.

DETD . . . mg/kg/d). When the tumors have grown to 1.0-1.3 cm diameter, the mice will receive an intravenous injection of 50-100 .mu.g anti-Ly6A.2/anti-**CD28** bispecific antibody (perhaps together with anti-Ly6A.2/anti-CD3 bispecific antibody if indicated by the studies in Section (h) above). Two or three. . . comparing the anti-tumor effects with those in mice which receive various control treatments, including unconjugated anti-Ly6A.2 Fab' and IgG, unconjugated anti-**CD28** Fab' and IgG, and anti-Class II immunotoxin alone.